1

BREAST CARCINOMA-ASSOCIATED FIBROBLASTS AND THEIR ADJACENT COUNTERPARTS DISPLAY TUMOR-ASSOCIATED FEATURES

Nahed M. Hawsawi, Hazem Ghebeh, Siti-Faujiah Hendrayani, Asma Tulbah, Maha Al-Eid, Taher Al-Tweigeri, Dahish Ajareem, Ayodele Alaiya, Said Dermime and Abdelilah Aboussekhra

King Faisal Specialist Hospital and Research Center, Department of Biological and Medical Research, MBC # 03, PO BOX 3354, Riyadh 11211, KSA

It has become clear that the genesis and thrive of carcinomas depend not only on genetic and epigenetic alterations in epithelial cells, but also on changes in the stroma. In order to identify these changes, we have undertaken cellular and molecular characterization of carcinoma-associated fibroblasts (CAFs) and their adjacent counterparts (TCFs) isolated from 12 breast cancer patients. Normal breast fibroblasts (NBF) from plastic surgery were used as normal control. While the α-SMA protein was undetectable in NBF cells, CAFs and TCFs were both positive for this myofibroblast marker. Furthermore, the p53/p21 response pathway to γ-rays was defective in 70% CAFs, whilst it was normal in all the TCF and NBF cells. In addition, the basal levels of p53 and p21 tumor suppressor proteins were lower in 83% of CAFs, and modulated in the majority of TCFs, as compared to NBFs. Interestingly, both TCFs and CAFs expressed high levels of the cancer marker survivin, and consequently exhibited high resistance to the killing effects of cisplatin and UV light. Moreover, most CAFs were positive for the proliferation marker Ki-67 and exhibited high proliferation rate as compared to NBF and TCF cells. Using the 2 dimensional gel electrophoresis technique, we have also shown that CAFs, TCFs and NBF present different proteome profiles, with many proteins differentially expressed between these cells, indicating that different genetic alterations occur in breast carcinoma-associated fibroblasts and also their corresponding adjacent counterparts.

2

INTRODUCTION TO RADIONUCLIDE THERAPY

Gregory P. Adams[1], Jorgen Carlsson[2] and Torgny Stigbrand[3]

[1]Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, BA, USA;
[2]Biomedical Radiation Sciences, Uppsala University, Sweden;
[3]Department of Immunology and Clinical Microbiology, University of Umea, Sweden

Targeted radionuclide therapy can provide an effective method of selectively focusing cytotoxic effects on tumor cells. These methods have been successfully employed in the clinical treatment of diffuse (liquid) malignancies but have, as of yet, failed to achieve similar successes in the setting of solid tumors. This talk will provide an overview of the successes of targeted radionuclide therapy, outline some of the hurdles that remain and discuss possible approaches that can be taken to enhance therapeutic outcomes.

3

OPTIMIZING ANTIBODY-BASED MOLECULES FOR RADIOIMMUNOTHERAPY AND RADIOIMMUNOIMAGING

Gregory P. Adams

Department of Medical Oncology, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111, USA

Due to prolonged retention in the circulation and restricted ability to penetrate into solid tumors, intact antibodies are often not the most effective vehicles for the delivery of radioisotopes to tumors for therapeutic and imaging applications. However, antibody-based molecules can be rationally modified to improve their ability to selectively deliver radioisotopes to tumor tissue. This presentation will review our experience and that of others on the impact of altering antibody size, affinity and ability to interact with FcRn on targeting efficiency.

4

CETUXIMAB WITH HEPATIC ARTERIAL INFUSION OF CHEMOTHERAPY FOR THE TREATMENT OF COLORECTAL CANCER LIVER METASTASES

B. Neyns, M. Aerts, Y. Van Nieuwenhove, C. Fontaine, L. De Coster, D. Schallier, J. Vanderauwera, F. De Munck, F. Vandenbroucke, H. Everaert, V. Meert, J. De Mey, M. De Ridder, G. Delvaux and J. De Grève

UZ-Brussel, Laarbeeklaan 101, 1090 Jette, Belgium

Background: Both hepatic arterial infusion (HAI) of chemotherapy and cetuximab (CET) have interesting activity for the treatment of colorectal cancer liver metastases (CRC-LM). *Patients and Methods:* Intravenous CET with HAI oxaliplatin (OXA) or *i.v.* Irinotecan (IRI) followed by HAI of infusion of folic acid modulated 5-fluorouracil 5-FU/l-FA was administered to patients (pts) with CRC-LM who had failed at least one line of prior chemotherapy. *Results:* Eight pts received *i.v.* CET with HAI-OXA (5 pts) and *i.v.*-IRI (3 pts) and HAI-5-FU/l-FA. Adverse events: repeated grade 3 skin toxicity (1 pt), abdominal pain with elevated liver enzymes and asthenia (2 pts), duodenal ulcer (2 pts) with catheter migration and intestinal bleeding (1 pt), reversible interstitial pneumonitis (1 pt), and cystic bile duct dilatation (2 pts) with

arteriobiliary fistulisation (1 pt). A partial response was documented in 5 pts (62%). The median time to progression was 8.7 months (95% confidence interval 8-14 months). *Conclusion:* Intravenous administration of CET with HAI of chemotherapy is feasible and has promising activity but is associated with specific toxicity.

5

PATHOLOGY AS A BRIDGE BETWEEN RESEARCH AND CLINICAL PRACTICE

Niki J. Agnantis

Department of Pathology, University of Ioannina Medical School, Greece

The word "Pathology" originates from the Greek words Pathos (suffering) and Logos (study) and, as its name implies, it is a discipline devoted to the study of the cause, the pathogenesis, the morphological changes and functional derangement of cells, tissues and organs in disease. Anatomic pathology has originated in Europe 245 years ago and it has come a long way since the time that Morgagni encouraged the postmortem search for the cause and nature of disease. During this long course the histological techniques have been continuously improving and pathologists have been incorporating a variety of methods in their every-day practice, making diagnosis more refined and definite. Today, pathologists are able to make diagnoses by examining a whole organ, a fragment of tissue, or even a few cells. Advances in facing cancer range widely, from basic research designed to understand the molecular causes of cancer, through the application of this knowledge for the patients' benefit. Both basic and clinical research, the latter being dependent on the former, are now developing at a fast pace. Pathology is the discipline that acts as a bridge between Clinical Medicine and Basic Sciences.

A classical paradigm on the role of the pathologist in basic research is his contribution in elucidating the pathogenesis of colorectal carcinoma. Every stage of adenocarcinoma development has been identified and the progressive accumulation of genetic changes at the molecular level has been shown to parallel the clinical and histopathologic progression defined as "adenoma-carcinoma sequence".

Another example is that of breast cancer. Subclassification of breast carcinomas is important, since some types such as tubular and medullary carcinomas, have better prognosis than others. However, the morphological features of the neoplasm do not always reveal its underlying biology, as patients with the same tumor type can demonstrate different courses of their disease. Several molecular markers have recently been developed to predict the response of neoplastic cells to a certain type of therapy. Immunohistochemical analysis of estrogen receptors α (ERα) and ERBB2/HER2/NEU expression can be used to predict responses to tamoxifen or aromatic inhibitors and trastuzumab/hesrceptin respectively, as these therapies are designed to target these molecules. FISH has proved to be a powerful molecular DNA technique in pathology. Another technique that is used with increasing frequency is CGH. New technology using DNA microarrays provides a systemic method to identify key markers for prognosis and treatment response by profiling thousands of genes expressed in a single tumor. Microarrays have the potential to revolutionize the practice of pathology by providing a molecular "signature" that is characteristic of each neoplasm.

All these new molecular pathology techniques are necessary, but they should be applied with caution. They should be critically appraised and analyzed in detail regarding cost/benefit, in order to contribute the most to patient care.

6

EFFECTS OF THERAPY WITH THE RADIOLABELLED SOMATOSTATIN ANALOGUE [^{90}Y-DOTA^{0},TYR^{3}]OCTREOTATE IN PATIENTS WITH MENINGIOMA: REVIEW OF 16 CASES

M. Agostini[1], F. De Lauro[1], M. Casi[1], V. Mattone[1], S. Severi[1], C. Fabbri[2] and G. Sarti[2]

[1]Nuclear Medicine Unit, Imaging Department, M. Bufalini Hospital, viale Ghirotti 286, 47023 Cesena;
[2]Health Physics, Imaging Department, M. Bufalini Hospital, viale Ghirotti 286, 47023 Cesena, Italy

Aim: Therapy using the radiolabeled somatostatin analog [^{90}Y-DOTA^{0},Tyr^{3}] octreotate (^{90}Y DOTA-TOC) (DOTA is 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid) has been used primarily in gastroenteropancreatic neuroendocrine tumors. Here we present the effects of this therapy in a small number of patients with meningiomas. In these patients the therapy of choice is surgery and radiotherapy, however, the presence of cellular structures for amine uptake and storage allow targeted therapy. Meningiomas are tumors derived from cap cells adherent to the dura mater, mostly close to the arachnoid villi or skull base foramina; they express different kinds of receptors. Meningiomas are frequently somatostatin receptor-positive, and somatostatin receptor scintigrafy may be used to differentiate remnant or recurrent meningioma from non-specific hyperperfusion during postsurgical follow-up. Treatment with ^{90}Y-labeled somatostatin analogs in patients with meningioma has been undertaken in selected cases. *Materials and Methods:* We evaluated 16 consecutive patients (7 male/9 female; mean age 64 yrs, range 39-78 yrs.) treated in our unit with ^{90}Y DOTA-TOC in the period 01-03-03 to 15-04-07. Eight patients received surgical treatments before nuclear medicine therapy, 4 had radiotherapy, 3 surgical treatments and radiotherapy and one patient no treatment. The mean cumulative administered activity for each patient was

6400 MBq (range 1480-12987 MBq), divided into 4 cycles. The interval between each single treatment was 6-9 weeks. Mean follow-up was 12 months (range 6-24 mo.). Renal scintigraphy with GFR study was performed before each treatment cycle. Routine haematology, liver and kidney function tests were applied prior to each therapy, as well as at follow-up visits. CT scan or MRI and somatostatin receptor imaging was performed within 3 months before the first therapy and 4-5 months after the last treatment to evaluate the effects of the therapy on tumour size and metastases. *Results:* Partial remission was found in 3 patients (19%), stable disease in 8 (50%) and progressive disease in 5 (31%). None of the patients presented bone marrow and renal toxicity after any treatment. The patient-assessed quality of life was judged as stable by 5 patients (31%), better by 6 (38%) and worse by 5 (31%). *Conclusion:* ^{90}Y DOTA-TOC can be effective in patients with meningioma. Response rates are lower than those in patients with gastroenteropancreatic neuroendocrine tumors. Most meningiomas were very large. Further studies are needed to confirm the treatment outcome because of the limited number of patients in our study. The side-effects of therapy are few and mostly transient, and neither renal nor marrow function seriously deteriorated in any of our patients.

7

GROWTH OF HUMAN TUMOUR CELLS IN A CELLULAR MICROENVIRONMENT SUPPLIED BY HUMAN EMBRYONIC STEM CELL INDUCED TERATOMAS

J. Cedervall[1], S. Jamil[1], B. Sveinbjörnsson[1], Kanter-L. Lewensohn[2], M. Eskandarpour[2], M. Gulyas[3], U. Ringborg[2], J. Hansson[2], P. Kogner[1] and L. Ährlund-Richter[1]

[1]Department of Woman and Child Health, and
[2]Department of Oncology-Pathology, Karolinska Institutet, 171 76 Stockholm;
[3]Department of Pathology and Cytology, Central Hospital, 80187 Gävle, Sweden

Objectives: For clinically relevant studies on tumour progression, *in vivo* experimental systems with a human cellular microenvironment would be advantageous. We have for this purpose studied growth support for adult *vs*. paediatric neuroectoderm-derived human tumours (melanomas and medulloblastomas/neuroblastomas), following injections into a predominantly species-specific environment consisting of human embryonic stem cell derived teratoma, induced in the mouse (the hEST-model). *Results:* A mature hESC-teratoma environment was permissive for the integration and growth of all the injected tumours (Cedervall *et al*. submitted), in line with previous results of Tzukerman *et al*., in a similar experimental system (Cancer Res, *66(7)*: 3792-3801, 2006). In addition, we found the resulting tumour histology similar to conventional xeno-graft models, with predominantly areas of densely packed tumour cells. Uniquely for the hEST-model, some tumours also showed areas with less dense growth appearing in a surrounding of loose mesenchymal or fibrous stroma. The latter tumour population, but not the former, showed markers and morphology indicative of dedifferentiation and migration. An enhanced neovascularisation was indicated in areas of human mesenchymal tissues facing the tumour growth. The results furthermore indicated a specificity in the process of integration, distinct for each tumour type. The unique experimental advantage of a human cellular microenvironment revealed species-specific interactions of the tumour cells with the surrounding microenvironment, as indicated by differential appearances of a selection of markers linked to differentiation/migration/malignancy. In conclusion, the hEST-model provides new exciting options for molecular *in vivo* studies on differentiation, invasiveness and malignancy, opening also possibilities for improved clinical relevance in studies and evaluations of novel therapeutic interventions.

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REGULATION OF FOCAL ADHESION TURNOVER BY ERBB RECEPTOR SIGNALING AND PRECLINICAL IMPLICATIONS FOR ERBB-2+ INVASIVE BREAST CANCER MODELS

Moulay Alaoui-Jamali

Segall Comprehensive Cancer Center, Lady Davis Institute of the Sir Mortimer B. Davis Jewish General Hospital, Departments of Medicine and Oncology, McGill University, 3755 Cote Ste-Catherine, Montreal, Canada H3T 1E2

An early event by which cancer cells switch from localized to invasive phenotype is initiated by the acquisition of motile properties; a process driven by dynamic assembly and disassembly of multiple focal adhesion (FA) and cell cytoskeleton proteins, which mediate cell–matrix attachments, extracellular matrix degradation, and can serve as traction sites for motile cells. These processes are regulated by the

activation of integrins, as well as by growth factor receptors, including the ErbB/Her tyrosine kinases. We previously reported that cancer cell invasion induced by overexpression of the ErbB-2 receptor is dependent on focal adhesion kinase (FAK), a major kinase and adapter protein of the FA signaling pathway. Here, we report that ErbB receptor signaling regulates FA turnover *via* the Src-FAK pathway. Using biochemical and confocal imaging assays, we demonstrated that selective inhibition of the Src-FAK signaling in a panel of ErbB-2-positive cells regulates FA turnover, leading to enhanced number and size of peripherally localized adhesions and inhibition of cell invasion. These phenotypes were not observed following inhibition of ErbB signaling in cells lacking Src or FAK, but are restored after re-expression of Src and FAK in these deficient cells. Furthermore, molecular studies on downstream events revealed that ErbB signaling regulates the turnover of several FA-associated proteins, including FAK, in part *via* increased calpain 2 activity. Immunohistochemical analysis on progression tissue microarray, composed of a large breast tissue bank from patients treated at this institution for various stages of breast disease, showed a correlation between overexpression of FAK and specific FAK-partners in FA signaling and cancer progression and outcome. The implications of these downstream targets for invasive breast cancer models and the impact on novel FA inhibitors in preclinical trials will be discussed.

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9

REAL TIME PCR (ReT-PCR): A NOVEL METHOD FOR THE DETECTION OF ESTROGEN RECEPTOR (ER) ALPHA AND BETA ISOFORMS AND THEIR VARIANTS

S.B. Al-Saji[1] and M.D. Al-Bader[2]

[1]College of Graduate Studies, Molecular Biology MSc Program;
[2]Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait

Introduction: Estrogen receptors -alpha and -beta mediate the actions of estrogens. Several mRNA splice variants exist for both receptors and the normal estrogen function results from a balance between the wild-type ERs and their functional variants that may interfere with the coexpressed wild-type forms in a dominant negative manner, or by becoming ligand-independently activated. In addition, ER-alpha and ER-beta isoforms can exert opposite biological activities, as it is evident that ER-alpha stimulates cell proliferation while ER-beta can inhibit ER-alpha-stimulated cell proliferation. *Methods:* 45 FFPE breast cancer samples were used in this study. ReT-PCR analysis was conducted using ER-alpha primer sets detecting wild-type (wt) and exon deleted 3, 5, 6 and 7 variants. The ER-beta primer sets used detected the wt ER-beta 1 and the ER-beta 2 and ER-beta 5 variants. At the end of the ReT-PCR cycles a dissociation curve (melting curve) was generated which showed the number of peaks for each sample at specific melting temperatures (Tm). If more than one peak is obtained at the higher melting temperatures then this indicates the presence of variants for the specific gene of interest. In addition the dissociation curves provide us with a peak derivative that reflects the intensity of the band. *Results:* Using this method minimal amounts of mRNA could be detected and many samples expressed not only the wt ER isoforms but also their variants. The Tm value served as a cut-off point for determination of wt *versus* variant ER expression. Wild-type to variant ratio was easily calculated from the peak derivatives. *Conclusion:* This method allowed us to detect both ER isoforms and their variants in FFPE breast cancer tissue. This method, by showing wt and variants, can be used as an invaluable tool in the clinical field to predict the response of patients to antiestrogen therapy.

10

EXPRESSION OF GROWTH HORMONE RECEPTOR IN NEOPLASMS OF THE PROSTATE

Anwar Al-Banaw[1] and David T. Lincoln[2]

[1]Faculty of Allied Health Sciences, Kuwait University, Kuwait;
[2]Entity Systems, Independent Research Foundation, Chapel Hill, QLD, Australia

Knowledge of the mechanisms initiating and regulating prostate neoplasm and mesenchymal–epithelial interaction during its development is limited and information about potential trophic agents incomplete. Apart from regulating the growth and functions of the normal human prostate gland, androgens are also involved in the growth of prostate cancer. In addition, receptors for steroid hormone, including insulin-like growth factor-I (IGF-I), have been shown to exert a regulatory effect in the normal prostate gland. Recent evidence indicates that growth hormone (GH) may play an important role in tumour cell growth. Somatostatin analogues have been found to retard the growth of experimental prostate carcinomas.

This study further investigates the expression of growth hormone receptor (GHR) in human benign prostate hyperplasia (n=20) and in prostate carcinomas (n=62) using immunohistochemical assays with a well-characterized monoclonal antibody (mAb 263) reactive against human GHR. Tumours, consisting of grade 1 (n=8), grade 2 (n=10), grade 3a (n=7), grade 3b (n=8), grade 4a (n=8), grade 5a (n=7), grade 5b (n=6), were classified according to the grading system of Gleason and based on the degree of glandular differentiation and the growth pattern of the tumour in relation to the stroma, as evaluated on low-power examination. To delineate tumour cell growth, immunohistochemical analysis of proliferating cell nuclear antigen, using PCNA polyclonal antibody, was used to investigate proliferative indexes.

Results from this investigation confirm the presence of specific receptors for GH in prostate tissue from patients affected by benign prostate hyperplasia (BHP) and carcinoma. GHR Immunoreactivity showed sub-cellular localization of the receptor in cell membranes, and cytoplasm and nuclei were also reactive in some cases. In cases of BHP, the GHR expression was localized throughout the epithelium of the tumour acini. Of the BHP investigated, 40% were weakly reactive with mAb 263. In prostate carcinomas, regular distinct expression of GHR was observed in the epithelium of the small, atypically formed glands in irregular arrangement. Heterogeneity of immunoreactivity was present with a variable range of positive cells. Of the 62 cases studied, 77% were moderately to strongly positive, In foci within higher Gleason grade and correlating with prostate-specific antigen (PSA), the relative proportion of positive cells and intensity of immunoreactivity was increased in higher grade carcinomas, compared to lower grades and BHP. In contrast to BHP and prostate carcinomas, GHR expression was absent in normal prostate epithelial cells. Furthermore, there was a positive correlation of GHR immunoreactivity with neoplastic cellular proliferation, as measured by PCNA, the percentage of PCNA-positive tumour cells, representing the average of 10 fields each tumour case was 1.97% in prostate hyperplasia; 6.3% for grade I; 9.6% in grade 2; 15.6% in grade 3a; 18.3% in grade 3b; 20.5% in grade 3c; 23.7% in grade 4a; 36.2% in grade 5a and 61.1% in grade 5b prostate carcinomas. In conclusion, the presence of GHRs in human BHP and carcinoma strongly supports the concept that GH, known to increase mRNA levels of androgen receptor, IGF-1 and IGF-1 receptors in immature prostate, reacts on prostate target tissue to facilitate cellular proliferation. Our results are consistent with the hypothesis that GH acts locally to generate IGF-1 which then acts as mitogenic factor for these cells. Whether these effects of GH on prostate tissue are mediated by a direct action of IGF-I in an autocrine mechanism regulating tumour cell growth remains to be investigated.

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ENDOTHELIAL CELL PROLIFERATION AND ANGIOGENESIS IN VASCULAR TUMOURS: A DIRECT ROLE FOR GROWTH HORMONE

Anwar Al-Banaw[1], Fred Sinowatz[2] and David T. Lincoln[3]

[1]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, Kuwait;
[2]Institute of veterinary Anatomy, University of Munich, Germany;
[3]Entity Systems, Independent Research Foundation, Chapel Hill, QLD, Australia

Introduction: Vascular tumours are common lesions of the skin and subcutaneous tissue, but also occur in many other tissues and internal organs. Well-differentiated tumours consist of irregular anastomosing, blood-filled vascular channels that are lined by variably atypical endothelial cells. Less differentiated tumours may show solid strands and sheets, resembling carcinoma or lymphoma. Several growth factors, including basic fibroblast growth factor, transforming growth factors and vascular endothelial growth factor, play a role in tumour angiogenesis. Growth hormone (GH) is mitogenic for a variety of vascular tissue cells, including smooth muscle cells, fibroblasts and endothelial cells and exerts its regulatory functions in controlling metabolism, balanced growth and differentiated cell expression by acting on specific membrane-bound receptors, which trigger a phosphorylation cascade resulting in the modulation of numerous signalling pathways and of gene expression. *Materials and Methods:* To address the site/mode of action through which GH exerts its effects in vascular tumours, a well-characterized monoclonal antibody, obtained by hybridoma technology from Balb/c mice immunized with purified rabbit and rat liver GH-receptor (GHR) and directed against the hormone-binding site of the receptor, was applied to total of 64 benign and malignant vascular tumours from different human organs to determine GHR expression. Quantitative immunohistochemical analysis of GHR expression, cycling nuclear protein (Ki-67) and proliferating cell nuclear antigen (PCNA) was carried out by calculating the percentage of stained cells using a Zeiss microscope connected to a computer with image analysis software. To ensure reproducible and objective assessment of staining, 10 representative areas, each containing 1,000 tumour cells, were observed under high-power field (objective lens x40) in a vertical section taken from the centre of the lesion. The proportion of positive cells was expressed as percentage of total cells counted. Grading of microvascular density (MVD) was according to a scoring system: up to 25 vessels = 1+, 26-50 vessels = 2+, 51-75 vessels = 3+, 76-100 vessels = 4+, while >100 vessels was graded as 5+. *Results:* Compared to their normal tissue counterparts, nuclear and cytoplasmic expression of GHR consistently resulted in strong receptor

immunoreactivity in the highly malignant angiosarcomas and Kaposi's sarcomas, and was localized in the cell membranes and cytoplasm, but strong nuclear immunoreactivity was also identified. The presence of intracellular GHRs is the result of endoplasmic reticulum and Golgi localization. Nuclear localization is due to identical nuclear GHR-binding protein. The receptor expression was especially prominent in the solid buds of newly forming capillaries of infiltrating vascular tumours, indicating a role of GH in tumour angiogenesis. There was a positive correlation of GHR expression and cellular proliferation and cycling, both Ki-67 and PCNA being significantly higher in vascular tumours with high GHR expression. The median grade MVD score of the vascular tumours varied from 2.4 for benign haemangiomas to 4.7 in highly malignant haemangiopericytomas. *Conclusion:* Our findings demonstrate that GHRs are strongly expressed in malignant vascular tumour cells. The importance of endothelial cells in angiogenesis and vascular tumour growth is emphasized by the positive correlation between vascular tumour cells having endothelial cell characteristics and both tumour vascularity and tumour growth rates. Malignant tumour cells, which are highly expressive of the receptor, have a greater proliferation rate compared to benign tumours. The presence of GHRs in endothelial cells of vascular neoplasm indicates that they are target cells and GH is of importance in the proliferation of vascular tumour angiogenesis. GH is necessary not only for differentiation of progenitor cells, but also for their subsequent clonal expansion and maintenance. This study supports the hypothesis that GH is involved in paracrine-autocrine mechanism, acting locally in regulating vascular tumour growth and will be useful for site-specific studies of the evolution of vascular cancer. The use of anti-GHR antibodies to block tumour progression is an intriguing possibility.

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MOLECULAR ANALYSIS OF CIRCULATING TUMOR CELLS (CTC): CLINICAL IMPLICATIONS

Tanja Fehm[1], Sabine Kasimir-Bauer[2], Erich Solomayer[1], Bahriye Aktas[2], Siegfried Hauch[3] and Winfried H. Albert[3]

[1]University of Tübingen, Department of Obstetrics and Gynaecology, Calwerstr. 7, 72076 Tübingen;
[2]University Hospital Essen, Department of Gynaecology, Hufelandstr. 55, 45122 Essen;
[3]AdnaGen AG, Ostpassage 7, 30853 Langenhagen, Germany

Background: Therapy is typically based on distinct properties of the primary tumor like HER2 or hormone status. However, metastases exhibit frequently a different phenotype leading to resistance towards therapy. The (re-)appearance of CTC may reflect this situation. *Objectives:* The studies were designed to evaluate the phenotype of CTC, to compare it with the one of the primary tumors in the same patients and to correlate the presence of CTC in metastatic breast cancer patients with the clinical outcome. *Methods:* Duplicate samples of 5.5 ml blood were drawn from patients and tested for the presence of CTC with the *AdnaTest BreastCancer* according to the manufacturer's instruction (AdnaGen AG) in a multiplex RT-PCR. This test reveals the over-expression of HER2, EpCAM and MUC1. The over-expression of the estrogen receptor (ER) and of the progesterone receptor (PR) was determined with the *AdnaTest ER/PR* in a separate multiplex RT-PCR using the same cDNA. The over-expression of HER2, ER and PR in the primary tumor was evaluated by IHC. *Results:* The primary tumors of 30 CTC positive patients were analysed for ERα and PR over-expression. 21 (70%) tumors were positive and 6 (20%) negative for both, ERα and PR. 2 (7%) tumors over-expressed ERα only. One tumor was negative for ER but positive for PR. 2 patients had ERα/PR positive, 22 (73%) negative CTC. 6 (20%) CTC were ERα positive but PR negative. None of the patients with an ERα/PR negative primary tumor developed positive CTC. Similarly, the primary tumors of 47 patients were analysed for HER2 over-expression. 7 (15%) cases were defined as triple positive, 40 (85%) as negative. However, 16 (40%) of the patients with HER2 negative tumors harboured HER2 positive CTC. The overall concordance of the histological findings on the primary tumors with the results obtained with the *AdnaTest BreastCancer* was 55% for HER2 and 50% for ER, strongly indicating that phenotypic changes may occur during the course of the disease. In a separate cohort of 32 metastatic patients therapy response was predicted in 78% of all cases. The persistence of CTC correlated significantly (p=0.005) with shorter survival. *Conclusion:* There is often HER2 over-expression on CTC in patients with HER2 negative primary tumors. This might offer the possibility to treat patients with Herceptin® who so far would not be eligible to it. On the other hand, the over-expression of ERα/PR is rarer on CTC, which might reflect resistance to hormone therapy. Testing for CTC may offer additional information with respect to prognosis, risk assessment for recurrence and prediction of therapy response.

13

IMPLICATION OF OXIDATIVE STRESS IN THE ANTITUMOR EFFECT OF TAXANES: FROM BASIC RESEARCH TO CLINICAL PERSPECTIVES

Jérôme Alexandre

Université Paris Descartes, Faculté de Médecine, EA 1833, Hôtel-Dieu, AP-HP, Paris, France

The taxanes (T), paclitaxel and docetaxel, are microtubule-targeted agents (MTA) widely used in cancer therapy. Their primary cellular effect is to cause abnormal stabilization of the dynamic microtubule polymerization. T induce failure of

mitosis but also alter intracellular signaling that involves microtubules.

Recently, oxidative stress has emerged as a major component of the intracellular signals controlling cell death and proliferation. We showed that paclitaxel (PCX) induces early hydrogen peroxide (H_2O_2) accumulation in human cancer cells. PCX cytotoxicity is inversely correlated to intracellular content of reduced glutathione (GSH), a key component of H_2O_2 scavenging. The GSH precursor, N-acetylcysteine, abolishes PCX antitumor activity in mice, confirming that H_2O_2 generation is a crucial step for T-induced cancer cell-death.

We showed that PCX promotes oxidative stress through enhancing the activity of NADPH oxidase (NOX) associated with plasma membranes. Treatment of breast cancer cells causes an increased translocation of Rac1 to the membrane fraction. Rac1 is a positive regulatory protein of NOX and may be associated with microtubules in cytosol. By activating NOX, PCX induces H_2O_2 accumulation outside the cells. Using co-culture systems, we observed that extracellular H_2O_2 causes lethal damage and proliferation inhibition to the bystander cancer cells not exposed to PCX. This may contribute to the anticancer activity of PCX. The bystander effect was also observed with other MTA but not with 5-fluorouracil or doxorubicin.

The superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion to H_2O_2, constituting a rationale to develop therapeutic combinations of T and SOD mimics. Mangafodipir, a contrast agent used in magnetic resonance imaging, has SOD-, catalase-, and GSH reductase-like properties, allowing it to act at multiple steps of the reactive oxygen species cascade. We observed that mangafodipir amplifies the inhibitory effect of PCX on tumor growth and protects mice against PCX-induced leucopenia and sepsis, improving its therapeutic index. This differential effect between normal and cancer cells may be related to the observation that, in cancer cells, basal H_2O_2 concentration is increased and antioxidant systems are overwhelmed.

These findings open important clinical perspectives. Expression of proteins controlling the cellular redox environment may influence the sensitivity of tumor cells to T and other anticancer agents. Finally, oxidative stress-modulating agents may improve the therapeutic index of T.

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LOW MOLECULAR WEIGHT PROTEIN TYROSINE PHOSPHATASE ISOFORMS AND CANCER PROGRESSION

Irina Alho, M. Clara Bicho, Raquel Carvalho, Alda Pereira da Silva, Luís Costa and Manuel Bicho

Genetics Laboratory, Metabolism and Endocrinology Center, Faculdade de Medicina de Lisboa, Av. Prof. Egas Moniz, Edifício Egas Moniz, P1C 1649-028 Lisboa, Portugal

Protein tyrosine phosphorylation is recognized as crucial for the generation of signals necessary for cellular metabolism, proliferation, growth, migration, and invasion of malignant cells. The contribution of protein tyrosine phosphatases (PTPs) for the control of cell phosphorylation state is as relevant as that of phosphotyrosine protein kinase.

Low molecular weight protein tyrosines (LMW-PTPs) are a family of 18 kDa enzymes that have been implicated in the regulation of cell growth without tissue specifity. Human red cell acid phosphatase (ACP1; EC 3.1.3.2) is a polymorphic enzyme member of the cytosolic LMW-PTPs: three common alleles (A, B and C) segregating at the ACP1 locus on the short arm of chromosome 2 (2p25) give rise to six genotypes. Each allele encodes two electrophoretically different isozymes, *fast* and *slow* (according to their relatively fast or slow anodal electrophoretic mobility), derived by alternative splicing of the primary RNA transcript and differing only in the sequence spanning residues 40-73. These isozymes are produced in allele specific ratios. These two isozymes may have different roles in the progression of oncologic pathology: *fast* are involved in migration, invasion and cell adhesion, activating different substrates after PDGF-R stimulation; *slow* isozymes, acting directly on PDGF-R, have growth factors as substrates, *e.g.* PDGF, leading to a decrease of cellular growth through its dephosphorylation.

ACP1 seems to have an oncogenic role through the increase of *fast* genotypes in cancer patients. *Fast* isozymes, associated with cytoskeletal organization, are the only ones activated by tyrosine phosphorylation in two positions of tyrosine after growth factor stimulus, which promotes adhesion and cellular migration and facilitates metastasis and invasion.

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A GENETIC LOOK AT MIDDLE EASTERN COLORECTAL CANCER

Khawla S. Al-Kuraya[1], Shahab Uddin[1], Maqbool Ahmed[1], Azhar Hussain[1], Fouad Al-Dayel[2] and Nasser Al-Sanea[3]

[1]Department of Human Cancer Genomic Research, Research Center at KFNCCC&R, [2]Department of Pathology, [3]Colorectal Unit, Department of Surgery, King Faisal Specialist Hospital and Research Center, P.O. Box 3354, Riyadh, 11211 Saudi Arabia

Colorectal Cancer (CRC) is a major cause of mortality and morbidity worldwide. In Saudi Arabia, the incidence of CRC is increasing, whereas the incidence rate was originally lower than in Western countries. According to latest statistics, CRC is considered the second most common cancer among Saudi males and the third most common among Saudi females. Significant improvements have been made in the management of this disease mainly through the introduction of adjuvant chemotherapy agents such as flurouracil and oxaliplatin. More

recently, advances in the understanding of tumor biology have led to the development of targeted therapies, allowing progress in the treatment of colorectal cancer.

The ubiquitin-proteasome system (UPS) regulates a number of intracellular proteins that govern cell cycle tumor, growth and survival *via* degrading a number of different polypeptides important for cell cycle progression and apoptosis. SKP2, an F-box protein targets cell cycle regulators, including cycle-dependent kinase inhibitor $p27^{Kip1}$, *via* ubiquitin-mediated degradation. SKP2 is frequently overexpressed in general types of cancer. We investigated the role of SKP2 and its ubiquitin-proteasome pathway in CRC using a panel of cell lines, clinical samples and a nude mouse model. Using immunohistochemical analysis on a large tissue microarray of 448 samples, an inverse association of SKP2 expression with $p27^{Kip1}$ protein levels was seen. A CRC subset with high level of SKP2 and low level of $p27^{Kip1}$ showed a decreased overall survival (p=0.0057). Treatment of CRC cell lines with bortezomib or expression of siRNA of SKP2 caused down-regulation of SKP2 and accumulation of $p27^{Kip1}$. Furthermore, treatment of CRC cells with bortezomib caused apoptosis *via* mitochondrial pathway and activation of caspases. In addition, treatment of CRC cells with bortezomib down-regulated the expression of XIAP, cIAP1 and survivin.

Finally, treatment of CRC cell line xenografts with bortezomib resulted in growth inhibition of tumors in nude mice *via* down-regulation of SKP2 and accumulation of $p27^{Kip1}$. Altogether, our results suggest that SKP2 and ubiquitin-proteasome pathway may be a potential target for therapeutic intervention for treatment of CRC.

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DOMAIN I OF THE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR IN SERUM IS AN INDEPENDENT PROGNOSTIC FACTOR IN NON-SMALL CELL LUNG CANCER

Charlotte E. Almasi[1], Ib J. Christensen[1], Gunilla Høyer-Hansen[1], Helle Pappot[1,2], Hendrik Dienemann[3] and Thomas Muley[4]

[1]The Finsen Laboratory, Rigshospitalet, Copenhagen;
[2]Department of Oncology, Rigshospitalet, Copenhagen, Denmark;
[3]Department of Thoracic Surgery, Thoraxklinik Heidelberg gGmbH, University of Heidelberg;
[4]Translational Research Unit, Thoraxklinik-Heidelberg gGmbH, University of Heidelberg, Germany

Introduction: The urokinase plasminogen activator (uPA) system is a cascade of reactions participating in the degradation of extracellular matrix during cancer invasion. The uPA receptor, uPAR, is a key enzyme consisting of three domains denoted I, II and III. In addition to its involvement in the plasminogen activation, uPA can cleave a neighbouring uPAR molecule between domains I and II. We have previously shown that high blood levels of both intact and cleaved uPAR forms are associated to short survival in patients operated for non-small cell lung cancer (NSCLC). *Purpose:* To validate the prognostic impact of intact and cleaved uPAR forms measured in serum from NSCLC patients. *Methods:* Serum sampled preoperatively was available from 171 patients radically operated for NSCLC (*population A*). The median observation time was 3.8 years. A subpopulation of 124 lung cancer patients was selected with squamous cell carcinomas (SCC) or adenocarcinomas (AC) in stage I-III (*population B*). This subpopulation was further restricted to those patients (n=90) having no other treatment than operation (*population C*). The levels of the different uPAR forms (intact+cleaved: uPAR(I-III)+(II-III), intact: uPAR(I-III), domain I: uPAR(I)) in the samples were measured by three in-house time-resolved fluoroimmunoassays. *Results:* Significant associations were found between both uPAR(I-III) and uPAR(I) and gender. uPAR(I) was the uPAR form with the most significant association to survival, and was therefore used for further analyses. High serum levels of uPAR(I) were associated to short survival in the three populations. These associations were independent of stage, histology, age, WHO performance status and therapy (*Population A*: HR=1.85, C.I.: 1.18-2,89, p=0.007; *Population B*: HR=2.03, C.I.:1.18-3.52, p=0.01; *Population C*: HR=3.05, C.I.: 1.47-6.34, p=0.003). In addition to uPAR(I), only stage was a significant prognostic factor in all three populations. No interactions between uPAR(I) and histological subtype could be detected. *Conclusion and Perspectives:* This study validates that uPAR(I) in serum is an independent prognostic factor in patients radically operated for NSCLC. A possible application of uPAR(I) is as a supplementary tool in the selection of early-stage NSCLC patients for adjuvant therapy.

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THE THIOREDOXIN-THIOREDOXIN REDUCTASE SYSTEM: OVEREXPRESSION IN THYROID CANCER

Fatma Al-Yatama[1], Maie Al-Bader[2] Fawziah M.A. Mohammed[1], Anwar G. Al-Banaw[1] and David T. Lincoln[3]

[1]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, Kuwait;
[2]Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait;
[3]Entity Systems, Independent Research Foundation, Chapel Hill, Australia

Oxidation-reduction has emerged as a fundamental biological control mechanism. One of the major redox control systems consists of thioredoxin (TRX) and thioredoxin reductase (TRX-

R). Together, they form a powerful system involved in many central intracellular and extracellular processes, including cell proliferation, redox regulation of gene expression and signal transduction, protection against oxidative stress, anti-apoptotic functions, growth factor and co-cytokine effects, and the regulation of the redox state of the extracellular environment. In recent times, this system has increasingly been linked to the development and expression of cancer phenotypes, cancer cells secreting thioredoxin in varied amounts. The secreted TRX tends to sensitize the cells to growth factors, produced by the cancer cells themselves, by increasing the potentiation of the growth factors, making the cells more susceptible to them and therefore leading to increased cellular proliferation. Thus, TRX acts like an enhancement for growth factors and stimulates the growth of cancer cells. In this investigation, we have used immunocytochemical approaches to simultaneously determine the expression and localization of both TRX and TRX-R in neoplasms of the thyroid gland. Thyroid cancer is the second most common malignancy following breast cancer in Kuwaiti females. The incidence of thyroid cancer per every 100,000 females is 7.4 and constitutes ten percent of all common malignancies. This retrospective study of thyroid cancers, involving 111 female and 55 male patients, consisted of benign colloid nodule (n=15), colloid goiter (n=14), multinodular goiter (n=21), papillary oncocytic neoplasm (n=10), follicular adenoma (n=16), follicular carcinoma (n=24), invasive follicular carcinoma (n=18) and papillary carcinoma (n=48), Immunohistochemical identification and localization of TRX was performed using purified mouse anti-human TRX monoclonal antibody. Expression of TRX-R was demonstrated using an anti-human TRX-R antiserum prepared by immunization of rabbits against purified human placental thioredoxin reductase. The antiserum was assessed for specificity by Western Blot analysis against purified human TRX-R and human cell and tissue extracts and detected as single 56K band of human TRX-R. The results from this investigation show increased staining intensity of both TRX and TRX-R in the cytoplasm and nuclei of thyroid cancer cells, compared to normal thyroid tissue. Expression of increased TRX immunoreactivity was found in 15% of the benign colloid nodule cases, in 45% of colloid goiters, in 52% of multinodular goiters, in 45% of papillary oncocytic neoplasm, in 30% of follicular adenomas, in 65% of the follicular carcinomas, in 72% of the papillary and in 85% of all invasive follicular thyroid carcinomas. Furthermore, increased levels of TRX immunoreactivity positively correlated with thioredoxin reductase (TRX-R) expression and localization. This enzyme, involved in the reduction of thioredoxin, was also highly expressed in thyroid cancer cells, reflecting that a large amount of its thioredoxin substrate is also present. Of the 166 thyroid cancer cases investigated, overexpression of TRX-R was found in 12% of the benign colloid nodule cases, in 40% of colloid goiters, in 50% of multinodular goiters, in 40% of the papillary oncocytic neoplasms, in 30% of follicular adenomas, in 52% of the follicular carcinomas, in 66% of the papillary and in 80% of all invasive follicular thyroid carcinomas. In the case of invasive follicular carcinomas, the majority showed a correlation between strongly positive thioredoxin and thioredoxin reductase expression, and number of positive lymph nodes. In conclusion, the correlation of TRX and TRX-R immunoreactivity with advanced malignancy suggests a positive association of enhanced expression of these two proteins with the more aggressive tumour phenotypes. Such tumours have a high proliferation rate, a low apoptosis rate and an elevated metastatic potential, all of which can be influenced by the actions of the thioredoxin–thioredoxin reductase system. Results from this investigation also indicate that thyroid tumour cells use thioredoxin as an autocrine growth stimulate. Occurrence of oxidative stress within the cells induces TRX-R release which, due to its anti-apoptotic activity, causes inhibition of apoptosis, resulting in abnormal cell proliferation initiating cancer development. Aggressive tumours display intense immunoreactivity of thioredoxin and thioredoxin reductase and due to the former, have a high proliferation rate and low apoptosis rate. This indicates that increased TRX and TRX-R expression is associated with tumourigenesis. In addition, secreted TRX can also act as an extracellular growth factor for both normal and tumour cells and enhance the sensitivity of the cells to other growth factors. As such, this study further emphasizes the potential benefits of anti-TRX/TRX-R agents in cancer therapeutics in the treatment of thyroid cancers.

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ASSESSMENT AND COMPARISON OF EFFLUX PUMPS OF CANCER CELLS AND MDR BACTERIA UNDER PHYSIOLOGICAL CONDITIONS BY A REAL-TIME SEMI-AUTOMATED SYSTEM

Leonard Amaral[1,2], Gabriella Spengler[1,2], Miguel Viveiros[1], Liliana Rodrigues[1,2], Ana Martins[1,2], Isabel Couto[1,3], Marta Martins[1,2], Séamus Fanning[4], Jean-Marie Pagès[5] and Joseph Molnar[6]

[1]Unit of Mycobacteriology, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (UNL), Lisbon, Portugal;
[2]UPMM, Instituto de Higiene e Medicina Tropical, UNL, Lisbon, Portugal;
[3]Centro de Recursos Microbiológicos (CREM), Faculdade de Ciências e Tecnologia, UNL, Caparica, Portugal;
[4]Centre for Food Safety, School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Dublin, Ireland;
[5]UMR-MD1, Transporteurs Membranaires, Chimiorésistance et Drug-Design Facultés de Médecine et de Pharmacie, Marseille, France;

[6]Institute of Medical Microbiology, University of Szeged H-6720, Szeged, Hungary

Assessment of overexpressed efflux pumps (EPs) is usually conducted with the common EP substrate fluorochrome ethidium bromide (EB) in the absence and presence of agents that are believed to inhibit EPs and hence promote the accumulation of EB. This method is conducted at room temperature with a buffer of pH 7 and usually without any source of metabolic energy. These conditions are far from those under which EPs are expected to function optimally. The semi-automated method to be presented utilises a buffer whose pH ranges from 5 to 8, contains a source of metabolic energy and is maintained at 37˚C. These are conditions that favour efflux and hence should be suitable for the study of EPs of multidrug-resistant (MDR) bacteria. Accumulation of EB, its efflux, and the effects of agents that increase accumulation and inhibit efflux, has been followed on a real-time basis with the aid of the Rotor-Gene 3000™ . The method has been applied for the study of EPs of MDR strains of *Escherichia coli*, *Salmonella, Enterobacter, Enterococcus, Staphylococcus* and mycobacteria. Overall, whereas at pH 5 CCCP, PAβN and phenothiazines do not increase the accumulation of EB or prevent its efflux under conditions that favor efflux, with increasing pH, these agents cause increased accumulation of EB, although the medium is highly supportive of efflux. The results suggest that at low pH, the energy (protons) that is used for driving efflux is supplied by the proton gradient, whereas at high pH the needed protons are provided by metabolic energy. Evaluation of the efflux pump of cancer cells was also conducted by the same methodology. The results presented show that the assessment and inhibition of efflux pumps of cancer cells that render the cells immune to cytotoxic agents can be conducted on a real-time basis with a degree of precision not possible with flow cytometry. Moreover, because of the capacity of the system, a large number of cell systems can be evaluated and compared with any one run of the method.

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AN ARTIFICIAL NEURAL NETWORK-BASED EVALUATION OF TUMOUR BIOMARKERS FOR THE PREDICTION OF NODAL SPREAD AND PROGNOSIS OF BREAST CANCER

Shirin Ameiryan, Gajanan V. Sherbet, Wai L. Woo and Satnam S. Dlay

School of Electrical, Electronic and Computer Engineering, University of Newcastle upon Tyne, UK

The presence of tumour cells in the regional lymph nodes is routinely employed to determine tumour spread and predict prognosis. Minimally invasive methods to achieve this have been keenly sought. Many biomarkers have been identified that appear to relate to the aggressive behaviour of cancer. This study aims to assess four biomarkers using an Artificial Neural Network (ANN) to predict the presence of metastatic tumour in the regional lymph nodes and to predict 5-year survival of patients with breast carcinoma. Evaluation of the impact of individual markers on predicting outcome and determining an optimum subset that can yield a high level of prediction accuracy in both cases is another objective of this study. The data set used for the analysis consists of four input markers viz. DNA ploidy, S-Phase Fraction (SPF), G0G1/G2M Ratio, oestrogen and progesterone receptor expression status (ER/PR) and two corresponding outputs to be predicted, one related to the nodal involvement and the other to 5-year survival of patients. The results indicate that amongst individual biomarkers, ER/PR provides the best accuracy of performance for both survival and nodal involvement of the tumour with 89% and 70% of accuracy respectively. Inspecting the accuracy outcome of different biomarker combinations for survival analysis, the best results have been obtained from the two biomarker subset consisting of SPF and G_0G_1/G_2M ratio which provided 89% prediction accuracy. In the case of nodal involvement prediction, the three marker subset containing DNA ploidy, ER/PR and G_0G_1/G_2M ratio demonstrated the highest accuracy which is 73%. Hence, for predicting prognosis in breast carcinomas, identifying two markers instead of all markers is sufficient to obtain more accurate prediction for survival analysis whilst the three-marker configuration is more suitable for nodal assessment. In addition, when all four markers were combined, the highest predictive accuracy is found to be 90% for both survival and nodal prediction. These findings suggest that ANN-based analysis commends itself as a highly accurate method for the prediction of lymph nodes metastases and prognosis.

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MECHANISMS OF RALT-DEPENDENT INHIBITION OF ERBB SIGNALLING

Sergio Anastasi

Laboratory of Immunology, Regina Elena Cancer Institute, Viale delle Messi D'Oro, 156-158 00158, Rome, Italy

RALT/MIG6/ERRFI-1 (hereafter referred to as RALT) is a transcriptionally-induced feed-back inhibitor of receptors belonging to the epidermal growth factor receptor family (EGFR/ErbB1, ErbB2, ErbB3 and ErbB4). Overexpression of RALT in cultured cells inhibits activation of ERK and AKT downstream to ErbB receptors and attenuates the mitogenic and transforming activity of ErbB oncoproteins. Conversely,

RNAi-mediated knock-down of RALT expression in EGF-treated cells causes a) extended duration of ERK and AKT activation; b) higher expression of G1/S cyclins; c) increased recruitment of cells into the mitotic cell cycle. *Ralt* null mice show a fully penetrant skin phenotype, characterized by aberrant proliferation of keratinocytes and enhanced sensitivity to skin carcinogens. Consistent with studies in cultured cells, skin lesions in *Ralt* null mice are reversed by Iressa, a clinically used inhibitor of the EGFR kinase. Thus, RALT is an essential negative regulator of ErbB signals and a potential tumour suppressor.

Herein we present our most recent work aimed at clarifying the molecular mechanisms which account for the essential role of RALT in the regulation of ErbB signalling. RALT binds to ligand-activated ErbB receptors *via* a region spanning aa. 325-375 (EBR, ErbB-binding region). The EBR is necessary and sufficient to inhibit the kinase activity of ErbB RTKs in *in vitro* assays, as well as in intact cells. Deletion mutagenesis studies indicate that the EBR binds to the –COOH lobe of the kinase domain of EGFR, a region known to be essential for the allosteric activation of the EGFR kinase induced by ligand-driven receptor dimerization. The EBR function is evolutionarily conserved, as the EBR module of the *D. rerio* RALT ortholog is capable of suppressing human EGFR.

Surprisingly, we observed that RALT-bound EGFR molecules undergo a seemingly normal endocytic traffic. This appears to contradict the consolidated notions that EGFR signalling is required for receptor down-regulation and inhibition of EGFR kinase blocks receptor endocytosis. We will present data supporting a role of RALT as effector of the endocytosis of kinase-suppressed EGFR. A model entailing a two-tiered mechanism of EGFR inhibition by RALT, namely kinase suppression and receptor down-regulation, will be discussed.

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FREE RADICALS AND CANCER

Jane Anastassopoulou

National Technical University of Athens, Chemical Engineering Department, Radiation Chemistry and Biospectroscopy, Zorgafou Campus, 15780 Zografou, Athens, Greece

It is well documented that lifestyle, environmental factors, exposure to chemicals, radiation (UV, X-rays γ-rays) and metabolic abnormalities can induce oxidative stress leading to the formation of free radicals, such as hydroxyl ($HO^{\bullet}$), hydroperoxyl anions ($O_2^{-\bullet}$), peroxyl ($OO^{\bullet}$), organic carbon radicals ($R^{\bullet}$), *etc*. Because of their very fast biochemical reactivity, free radicals can damage directly or indirectly DNA and then this damaged DNA, when in excess, can cause mutations, which alter cell signalling pathways creating cancer. The primary sites of attack are the heterocyclic purine and pyrimidine bases. Several investigations, *in vitro* and *in vivo*, have shown that the most important reaction in these cases is the addition of hydroxyl free radicals to imidazole rings of DNA, producing 8-OHdG, which also is used as a biomarker. It was found that the amount of 8-OHdG is higher in tissues of women with breast cancer than to those with no cancer. Free radicals can also induce DNA strand breaks, single (ssb) or double (dsb), which are characterized by the 3-phosphoglycolate-ended fragments.

We have used micro-Fourier Transform Infrared spectroscopy, an easy-to-use and non destructive technique, to investigate the very early-stages of breast cancer through the infrared spectra. Considerable changes were observed in the spectra in the region 1650-1500 cm^{-1} of amide I and amide II absorptions, as well as in the region of 1200-900 cm^{-1}, where the phosphate groups of DNA absorb. Spectral analysis allows us to conclude that mapping of the spectra of breast tissues could give us information about damages in the tertiary and secondary structures of proteins and in particular the functional groups of biological molecules, which are indicative of early-stages of development of cancer.

For the early diagnosis (pre-diagnosis) and therapy of breast cancer it is crucial to develop a non-destructive bioanalytical technique, such as micro-FT-IR spectroscopy, to obtain images of the breast, which are independent of breast shape and mass density in order to detect lesions, which are difficult to scan with the existing techniques.

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DNA TOPOISOMERASES – CELLULAR TOOLS WITH HUGE IMPACT ON GENOME STABILITY

Kamilla Kristensen, Jakob M. Pedersen, Simon Bendsen, Lotte Andreasen, Birgitta R. Knudsen, Lotte Bjergbaek and Anni H. Andersen

Department of Molecular Biology, University of Aarhus, C.F. Møllers Allé, Bldg. 130, DK-8000 Aarhus C, Denmark

DNA topoisomerases are ubiquitous enzymes which have evolved to solve the topological problems generated whenever the two DNA strands are separated to expose the encoded genetic information. This is the case during DNA transcription, where the separation of the strands is local and transient, and during DNA replication, where the separation is permanent.

Two types of topoisomerases exist, type I, including eukaryotic topoisomerase I and type II, including eukaryotic topoisomerase II. The enzymes remove topological problems manifested as changes in the number of windings in the DNA double helix, DNA interlinks, and DNA knots. Type I enzymes operate by introducing a transient cleavage in one of the DNA strands, whereas type II enzymes introduce a transient double-

strand break. During the DNA cleavage event, the enzymes become covalently linked to the generated DNA ends and topological structures are solved by passage of intact DNA through the breaks. After DNA passage, the breaks are resealed and the enzymes leave the DNA or go through a new catalytic cycle.

The covalently linked topoisomerase-DNA complex generated as an intermediate in the catalytic cycle of DNA topoisomerases presents a potential danger to the cell as the breaks can become permanent if DNA tracking machineries collide with the complexes. Balanced enzyme activities and efficient repair systems are therefore crucial to avoid genomic instability as a cause of topoisomerase action. Advantage is taken of the intermediate topoisomerase-DNA complexes by antitumor agents targeting DNA topoisomerases. These drugs change the enzyme into a cellular poison by stabilizing the complex, the result being chromosomal fragmentation and cell death. Lack of topoisomerase activity is another cause of genomic instability. In this case accumulating, unresolved topological structures may act as roadblocks and result in DNA breaks during replication and transcription.

We are currently investigating the role of DNA topoisomerases for global gene expression and the impact of the enzymes for genome stability during replication. Aspects of topoisomerase action and biological functions, as well as implications for genomic stability will be discussed.

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S100A4 IN MAMMARY GLAND BRANCHING MORPHOGENESIS

Kristin Andersen[1], Hidetoshi Mori[2], Jimmie Fata[3], Gunhild Mælandsmo[1] and Mina Bissell[2]

[1]Department of Tumor Biology, Institute for Cancer Research, Norwegian Radium Hospital, Oslo, Norway;
[2]Life Science Division, Lawrence Berkeley National Laboratory, CA;
[3]University of New York, College of Staten Island, NY, USA

S100A4, also called FSP1, belongs to the S100 family of small (10-12 kDa), calcium binding proteins. The members in this family have no known enzymatic activity, but undergo conformational changes during calcium binding, opening hydrophobic domains responsible for binding target proteins. S100 proteins are expressed both intra-, and extracellularly, in a cell- and tissue-specific manner. The evidence linking the expression of S100A4 in cancer cells to increased metastatic capacity is, after almost 20 years since its discovery, quite convincing. In addition, numerous publications on clinical material from several types of cancer have confirmed the association between S100A4 expression in primary carcinoma cells and a more severe prognosis. This was previously demonstrated in a panel of 349 early-stage breast cancer biopsies, where expression of S100A4 was a stronger prognostic marker than both lymph node infiltration and hormone receptor status. Interestingly, both the intracellular and extracellular version of the protein have been coupled to increased metastatic capacity of cancer cells. Both versions have been shown to induce MMP expression, and studies suggest that extracellular S100A4 activated NF-κB through an as yet unknown receptor.

The mammary gland undergoes extreme morphological changes throughout puberty. Hormones trigger the epithelial cells to proliferate and invade the mesenchyme, giving rise to a defined tree-like structure of ducts, with complex tissue architecture, in a highly regulated branching morphogenesis. Interestingly, the highly controlled branching morphogenesis and the metastatic spread of breast carcinoma cells have several similarities, they both require cell proliferation and invasion of the surrounding extracellular matrix. In this study, our goal was to elucidate the expression of S100A4 in normal breast tissue and to investigate whether S100A4 has a role in the normal mammary development. Mammary glands from mice and humans were analyzed for *in vivo* S100A4 protein expression by immunohistochemistry and RT PCR, respectively. Furthermore, organotypic 3D cultures of primary mouse mammary epithelial cells, and mammary epithelial cell lines from mice, were employed as functional model systems. We found S100A4 expressed in some mammary epithelial cells and its mRNA expression peaked during the period of ductal elongation in mice mammary gland. Using 3-dimensional organotypic *in vitro* models and shRNA, we furthermore demonstrated that both extracellular and endogenously expressed S100A4 up-regulated MMP expression, and contributed to a branching phenotype in normal epithelial cells. We propose the stimulatory effects of S100A4 on branching morphogenesis as an explanation as to why S100A4 promotes a metastatic phenotype in early-stage mammary carcinoma cells.

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CROSSTALK BETWEEN ADHESION MOLECULES AND TUMOR PROGRESSION

Alexandra Canonici[1], Wim Steelant[2], Véronique Rigot[1], Erik Bruyneel[4], Frans Van Roy[5], Françoise Garrouste[1], Gilbert Pommier[1] and Frédéric André[1]

[1]INSERM UMR 911, Aix-Marseille Université, Marseille, France;
[2]New Mexico Tech, Laboratory of Biochemical and Biomedical Research. Socorro, NM, USA;
[3]Department of Experimental Cancerology, Ghent University Hospital, Ghent;
[4]Department for Molecular Biomedical Research, VIB - Ghent University, Ghent, Belgium

Dynamic crosstalk between cell adhesion molecules, extracellular matrix and soluble informative factors is essential for cancer cell migration and invasion. Here, we investigated the mechanisms by which the E-cadherin/catenin complex and αv integrin can modulate insulin-like growth factor-I (IGF-I)-induced cell migration. Human colon mucosa, human colon cancer cell lines, HT29-D4 and HCT-8 derivatives that differ in their expression of α-catenin were used as models. Interactions between E-cadherin, αv integrin and IGF-I receptor (IGF-IR) were analyzed by co-immunoprecipitation and immunolocalization experiments. The impact of these interactions on cell mobility was determined by haptotaxis assays. We report that αv integrin, E-cadherin and IGF-IR form a ternary complex in both cultured cancer cells and human normal colonic mucosa. Alpha-catenin regulates the scaffolding of this complex. IGF-IR ligation by IGF-I induces the disruption of the complex and the relocalization of αv integrin from cell–cell contacts to focal contact sites. This perturbation is correlated with the observed increase in cell migration. These results suggest that regulation of the αv integrin/E-cadherin/IGF-IR scaffolding is essential for the modulation of cell mobility. Its alteration could be of major importance to sustain alterations in cell adhesion that occur during cancer cell invasion and metastasis.

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ADVANCES IN THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

E. Andreadou

Department of Neurology, Athens National University, "Aeginition" Hospital, Athens, Greece

Motor neurone disease or amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that specifically affects upper and lower motor neurones (MNs) and causes loss of power and function of skeletal muscles, with an annual incidence 0.3-2 per 100,000 population. The course of the disease is relatively rapid, with an average duration of around 3.5 years. Death is most frequently due to respiratory failure. Although the cause of ALS remains unknown, several pathways have been implicated in disease pathogenesis including glutamate mediated excitotoxicity, mitochondrial dysfunction, neuro-inflammation, apoptosis, oxidative stress, protein aggregation, aberrant axonal transport, and autoimmunity.

The management of patients with ALS has changed rapidly over the past 20 years. Although ALS is incurable, it is treatable. Advances in understanding the biology of ALS has led to the development of one marketed treatment, improved clinical management of people with ALS, and many clinical trials of novel therapies. This lecture will focus on recent advances in the development of new pharmacological agents, the combination of drug therapies, the new promising treatment strategies such as the transplantation of stem cells for the replacement of the damaged or lost MNs and the symptomatic care of patients with ALS.

Until now, the only drug that has shown evidence of neuroprotective effects in clinical trials in MND is riluzole. The drug has only marginal effects on survival and quality of life of ALS patients, as it prolongs survival by an average of only 3-4 months. Although it is considered an antiglutamate agent, its mechanism of action is obscure. In a recent study we have confirmed the lower rate of disease progression after treatment with riluzole, both in the spinal and the bulbar subtype of ALS, without any significant impact on plasma levels of the aminoacids glutamate and glycine. Our results possibly indicate additional mechanisms of action of the drug, besides its antiglutamatergic properties. Although many other agents have been tried in ALS without clear benefit, several new promising therapies are under development. Encouraging data are available from human studies for several agents including talampanel, tamoxifen, sodium phenylbutrate, arimoclomol and lithium. However, recent results suggest that addressing multiple components of ALS pathology in combination therapies might be the most effective way of tackling the disease. Combinations of anti-inflammatory and anti-excitotoxic drugs as treatments in animal models of ALS (mSOD1 mice) have been shown to be superior to application of their single components alone. Recently, in a face II trial the celecoxib-creatine combination was selected as preferable to the minocycline-creatine combination for further evaluation, although each of the three drugs individually has failed a phase III trial in ALS.

Other therapies besides the administration of pharmacological agents include viral vectors for gene delivery, other therapeutic factors that reduce endogenous motor neuron loss, minimize reactive astrocytosis and enhance connectivity of new neurons with host circuitry, as well as training regimens that modify these new spinal cord circuits. Moreover, transplantation of stem cells (adult, embryonic or neural) offers an intriguing strategy for slowing disease progression and/or promoting recovery of function because engrafted cells have the potential of replacing lost or dysfunctional neuronal and glial cell types as well as non-neural elements which contribute to recovery. Stem cell grafts can also provide additional benefits, including neuroprotection, modification of the immune response, reprogramming of endogenous stem cells, generation of new bridges or lost circuitries and delivery of therapeutic factors such as neurotrophic proteins and missing gene products. However, stem cell therapy remains entirely speculative at present. It is uncertain whether cell-based therapies will succeed without firstly interrupting the systemic disease process. Therefore progress in the field will accompany understanding of the underlying disease-specific

pathways and cell-replacement may need to be combined with immunosuppressant or neuroprotective approaches.

More realistically at present, a multidisciplinary approach can prolong survival and improve or maintain quality of life of patients with ALS. Disease progression gives rise to severe disability, respiratory impairment, speaking and swallowing difficulties.

The management of respiratory impairment in patients with ALS comprises ventilatory support. Assisted non-invasive ventilation is usually provided by a bilevel positive pressure device (BiPAP) and is a major advance in the management of ALS, as it has a survival benefit much greater than that of riluzole. Starting non-invasive ventilation is recommended when patients with ALS have symptoms related to nocturnal hypoventilation, frequently with dyspnoea and orthopnoea. Tracheostomy ventilation is an option when non-invasive ventilation is not able to compensate for respiratory impairment; however it is beyond the means for most patients. Swallowing difficulties lead to malnutrition and weight loss. Since nutritional status is a risk factor for survival, nutritional support is essential to the care of patients with ALS. Enteric feeding maintains good nutrition and hydration, stabilises weight, provides a way to give drugs, and might improve quality of life. Enteric feeding can be done by nasogastric tube, percutaneous endoscopic gastrostomy, or radiologically inserted gastrostomy. Patients with ALS also develop deficits that impair their ability to communicate. Development of brain-computer interfaces that provide connection between the brain and a computer can help to restore communication in severely impaired patients, through voluntary regulation of brain activity as a response to sensory stimulation.

Despite remarkable progress in understanding the underlying biology, ALS is a progressive, devastating and ultimately fatal disease and all patients will reach the terminal phase of respiratory insufficiency, inadequate nutrition and hydration and severe psychological distress. Consequently, there remains a critical need to develop additional treatments that will slow disease progression and ultimately turn ALS into a long-term treatable illness. Until more effective, disease-modifying therapies will be developed, improved clinical standards of care will play a major role in survival and quality of life of patients with ALS.

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MULTIPLE PATHS TO DRUG RESISTANCE PHENOTYPE IN CHRONIC MYELOID LEUKEMIA PATIENTS UNDERGOING IMATINIB MESYLATE TREATMENT

Ravindran Ankathil, Mohd Khairi Zahry, Rosline Hassan, Abu Dzarr, Hoh Boon Peng and Abdul Aziz Baba

School of Medical Sciences, Health Campus, University Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia

The introduction of Imatinib mesylate (Gleevec/Glivec) to the therapeutic armamentarium has changed the current management of Chronic Myeloid Leukemia (CML) patients. Despite being the first line treatment for CML, resistance to Imatinib is emerging as a real clinical problem in the management of CML. Therapeutic resistance to Imatinib may be primary or secondary. Resistance development is a multifactorial phenomenon in patients with CML, mediated by a diversity of mechanisms. There are two broad mechanisms of resistance (1) BCR-ABL dependent and (2) BCR-ABL independent. BCR-ABL dependent pathways include ABL kinase domain mutations and BCR-ABL amplification. Data on cytogenetic and molecular response of 42 CML patients treated with Imatinib, at Hospital University Sains Malaysia and the data on dHPLC based mutation analysis performed on 16 CML patients showing signs of disease resistance will be presented. BCR-ABL independent pathways may include several mechanisms. Various genetic and epigenetic alterations are proposed as candidate mechanisms involved in BCR-ABL independent pathways to IM resistance in CML patients and these multiple paths to IM resistance phenotype will also be discussed.

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SAHA ANTICANCER ACTIVITY THROUGH DEREPRESSION EFFECT ON CRITICAL GENES IN HPV CELL LINES

Gabriela Anton[1], Lorelei I. Brasoveanu[1], Anca Botezatu[1], Iancu Iulia[1], Stoian Mihai[1] and Natalia Cucu[2]

[1]Stefan S. Nicolau -Institute of Virology, Bucharest; [2]University of Bucharest, Romania

The interaction between DNA and histones is crucial for modulating the accessibility of transcription factors to DNA regulatory sequence. The chromatin structure is important for transcription regulation, the balance between active or silenced status being coordinated by the activity of enzyme effectors involved in chromatin remodeling, which modify DNA (DNA methyltransferase – DNMT) and histones (histone acetyltransferase – HAT and histone deacetylase – HDAC). New anticancer drugs are therefore currently targeting these epigenetic factors by their inhibitors, aiming at reactivation of the aberrantly silenced tumor suppressor genes. Current experimental and epidemiologic data confirmed that the human papillomavirus (HPV) is the causal agent in the development of cervical carcinoma. Taking into account HPV role in carcinogenesis, the aim of our study was to investigate the antitumor effect of SAHA (suberoylanilide hydroxamic acid, a histone deacetylase inhibitor) in cell culture models. HPV immortalized cell lines CaSki and Hela and a low risk HPV cell line obtained from a cervical xenograft, were treated with increasing doses of SAHA (0.5-2.5 μM) for 24 through

48 h. We noticed that SAHA has an antitumor activity by blocking cell proliferation and inducing tumor cell apoptosis in HeLa and CasKi cell lines. p21(WAF1) and p53 expression levels were higher at 2.5 μM/24h SAHA as appreciated in real-time PCR. mRNA levels of Dnmt1 were slightly increased at the same concentration of SAHA and were appreciated in the same conditions. By contrast, in lrHPV line, DNMT1 activity seems to increase at 2.5 μM SAHA after 48 of SAHA treatment. An interestingly effect upon DNA methyltransferases has been also observed. While DNMT1 mRNA levels slightly increased, DNMT3b immunoreactivity presented a rather constant feature. The only affected enzyme was DNMT3a whose immunoreactivity decreased significantly at high SAHA concentrations.

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SIMPLE STRUCTURAL CHROMOSOMAL ABNORMALITIES IN OVARIAN CANCER

Xristos Aravidis[1], Anna D. Panani[1], Zoi Kosmaidou[2] and Aristides Antsanklis[3]

[1]The Critical Care Department, Medical School of Athens University, Cytogenetics Unit, Evangelismos Hospital, Athens;
[2]Department of Genetics, Alexandra Maternity Hospital, Athens University, Athens;
[3]First Department of Obstetrics and Gynecology, Alexandra Maternity Hospital, Athens University, Athens, Greece

Ovarian cancer represents the leading cause of death among patients with gynecological cancer. The identification of chromosomal abnormalities is a useful strategy toward understanding tumorigenesis and specific chromosomal associations. Since single chromosomal changes might be primary events implicated in the initiation of the neoplastic process, the aim of the present study was to investigate the presence of simple structural chromosomal changes in ovarian cancer. Reviewing samples of ascitic effusions cytogenetically studied in our laboratory by direct culture of tumour cells and a G-banding technique, we found two cases with a diagnosis of ovarian cancer, which presented simple chromosomal abnormalities. The first case presented an abnormal clone of cells with an acquired pericentric inversion of chromosome 9, inv(9)(p11q13), as a sole anomaly. The second case presented simple chromosomal changes with involvement of the Xq23 chromosomal region, while a translocation t(X;11)(q23;q23) was also defined. The significance of the acquired pericentric inversion 9 in the development of the neoplastic process remains unknown. It is necessary, the chromosomal regions Xq23 and 11q23 to be molecularly further investigated in ovarian cancer. The documentation of more ovarian cancer cases with simple chromosomal abnormalities is considered of major importance facilitating the identification of candidate genes involved in the neoplastic process.

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TAUROLIDINE AND HONOKIOL: NOVEL POTENTIAL ANTI-NEOPLASTIC AGENTS FOR OSTEOSARCOMA THERAPY?

Matthias J.E. Arlt, Denise K. Walters, Patrick Steinmann, Ingo J. Banke, Roman Muff, Walter Born and Bruno Fuchs

University Hospital Balgrist, Orthopaedic Research, Forchstrasse 340, 8008 Zurich, Switzerland

Osteosarcoma (OS) is the most common primary bone cancer and pulmonary metastasis the leading cause of death in OS patients. The currently used chemotherapeutics exhibit severe, toxic side-effects which may cause life-threatening conditions. Thus, the development of novel agents with anti-metastatic potential and reduced toxicity is essential. Honokiol, an extract of the Magnolia tree, has recently been shown to have anti-neoplastic properties against several malignancies including multiple myeloma, breast, gastric and prostate cancer. Taurolidine (active agent of Taurolin®) is a well-known broad spectrum antibiotic that has been used for over 15 years for the treatment of severe surgical infections. It has also recently been shown to possess anti-neoplastic properties against a variety of human cancers. Therefore, in the present study, we investigated the therapeutic potential of these two novel anticancer drugs in our OS models. *In vitro*, both compounds showed pro-apoptotic and growth-inhibitory activity in a dose-dependent manner against all 9 human and 2 murine osteosarcoma cell lines tested. The IC_{50} values ranged between 8 and 16 μg/ml for honokiol and between 19 and 64 μM for taurolidine. Subsequently, we examined the effects of honokiol and taurolidine on OS metastasis in our two syngeneic OS mouse models. Highly metastatic, *lacZ*-tagged LM8 and K7M2 OS cells were injected *s.c.* into C3H mice and *i.v.* into BALB/c mice, respectively. Treatment with honokiol (3 mg/mouse/day) significantly reduced lung and liver metastases by 41% to 75% in both OS models. In contrast, treatment with taurolidine (15 mg/every other day) enhanced experimental K7M2 lung (1.7-fold) and liver (up to 50-fold) metastasis. Also in the LM8 model, taurolidine treatment significantly increased spontaneous LM8 lung (up to 2.8-fold) and liver (up to 20-fold) metastasis. Interestingly, dose-dependent severe hepatic deformations and atrophies could be observed in tumor-bearing as well as healthy mice upon treatment with taurolidine. Histological examinations revealed that liver lesions consisted of a multifocal fibrous thickening of the liver capsule, which was most pronounced in the strongly atrophic left median liver lobe. The liver deformations were accompanied by up to 10 times increased blood serum levels of the liver parameters ALT, AST and GLDH in the taurolidine

treated mice. These data indicate for the first time an unexpected, lung and liver metastasis promoting activity of taurolidine, accompanied with severe liver deformations. These organ-specific side-effects need to be taken into account regarding clinical introduction of taurolidine in cancer treatment. On the other hand, the natural compound honokiol was shown to possess potent antineoplastic and antimetastatic activity against osteosarcoma cell lines and may have potential as a novel OS chemotherapeutic agent.

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SCINTIGRAPHY WITH ^{99m}Tc- TEKTROTYD IN THE DETECTION OF NEUROENDOCRINE TUMORS

Vera Artiko, Vladimir Obradović, Nebojša Petrović, Dragana Šobić, Smiljana Pavlović, Bojana Popović, Djuro Macut and Svetozar Damjanović

Institute for Nuclear Medicine, Institute for Endocrinology, Diabetes and Metabolic Diseases, CCS, Belgrade, Serbia

Aim: The aim of the study is the detection of neuroendocrine tumors with ^{99m}Tc-Tektrotyd, a radiopharmaceutical indicated for the diagnosis of tumors with overexpression of somatostatin receptors. *Patients and Methods:* Whole body scintigraphy was performed in 66 patients up to 24h after *i.v.* administration of 740MBq ^{99m}Tc-Tektrotyd, as well as SPECT of particular regions. *Results:* From 18 patients with neuroendocrine tumors of unknown origin there were 14 true positive findings (TP) (8 with liver metastases, 6 with lung metastases, 4 with bone metastases and one with mediastinal gland metastases), 4 false negative findings (FN) (2 with liver metastases of the poorly differentiated tumors, and two second with very small lung metastases <1 cm). In 12 patients scintigraphy contributed to the further management of the patients. In the group of 16 patients with gut carcinoids there were 8 TP (6 with liver metastases), 4 true negative findings (TN) (after surgery), 2 FN (after surgery, small lung metastases <1 cm) and 2 FP (physiological accumulation of the activity in the bowel). In 8 patients scintigraphy contributed to the further management of the patients. In the group of 14 patients with neuroendocrine pancreatic carcinomas there were 8 TP (6 with liver metastases and one with metastases in paraortal lymph nodes) and 6 TN (somatostatinoma, insulinoma and carcinoid after surgery). In 6 patients scintigraphy contributed to the further management of the patients. In the group of 12 patients with lung carcinoids there were 8 TP (4 with liver, 2 with lung metastases and 2 with bone metastases), 2 TN (after surgery) and 2 FN (poorly differentiated). In 4 patients scintigraphy contributed to the further management of the patients. In the group of 6 patients with gastrinomas (jejunal, paraduodenal and pancreatic) there were 4 TP findings and two TN. In 4 patients scintigraphy contributed to the further management of the patients. Overall, the sensitivity of the method is 84%, specificity 88%, positive predictive value 95%, negative predictive value 64% and accuracy 85%. *Conclusion:* These preliminary results show that scintigraphy of neuroendocrine tumors with ^{99m}Tc-Tektrotyd is a useful method in diagnosis, staging and follow up of the patients suspected to have neuroendocrine tumors. It is also helpful in the appropriate choice and monitoring of the therapy, including radionuclides.

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THERAPY OF NEUROENDOCRINE TUMORS WITH ^{90}Y DOTA TATE –FIRST RESULTS

Vera Artiko, Vladimir Obradović, Nebojša Petrović, Dragana Šobić-Šaranović, Divna Đokić, Drina Janković, Nadežda Nikolić, Bojana Popović and Svetozar Damjanović

Institute for Nuclear Medicine, Institute for Endocrinology, Diabetes and Metabolic Diseases Clinical Center of Serbia, Laboratory for radioisotopes "Vinča", Institute for Nuclear Sciences Vinča, Serbia

Aim: Preliminary results of the therapy of NETs with ^{90}Y DOTA TATE (Polatom, Poland) are presented. *Patients and Methods:* We investigated 15 patients with various neuroendocrine tumors. In all of them, together with other laboratory analyses and imaging methods, scintigraphy with somatostatin analogues was performed (in 3 with ^{111}In Octreoscan and in the other 4 with ^{99m}Tc Tektrotyd) and a high tumor was uptake observed. The therapy was performed with 2-4,5 GBq ^{90}Y DOTA TATE per patient per one cycle, by slow infusion in physiological liquid (150 ml/15 min).Between the cycles, there was a time delay of 6-8 weeks. Thirty min before therapy, patients began receiving an infusion of amino acids (arginine and lysine) for 4h. Before that, all therapies with somatostatin analogues were withdrawn. 24h-96 h after therapy, "bremsstrahlung" whole body imaging, SPECT and particular planar images were performed with a gamma camera. Results: Analysis of the "bremsstrahlung" images showed uptake of the radiopharmaceutical in the liver, but the most of the activity was observed in the regions of the "hot spots" registered with previous ^{99m}Tc Tektrotyd and ^{111}In Octreoscan images. According to our results, after therapy, in two patients progressive disease (PD), in seven stable disease (SD), and in six partial remission (PR) occured. Up to now, there were no major clinical side-effects in the hepatic function. Transient pancytopenia occurred in two patients, and impairment of kidney function in one. *Conclusion:* In spite of insufficient data, beneficial effects on clinical symptoms, hormone production and tumor proliferation were found, without major clinical side-effects. Thus, according to these preliminary results, treatment with ^{90}Y DOTA TATE is a feasible method and might be useful for the management of patients with inoperable or disseminated neuroendocrine tumors.

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SETUP AND CHARACTERIZATION OF ANIMAL MODELS OF HEAD AND NECK CANCER IN IMMUNOCOMPETENT RATS

Karine Aubry[1] and Michel Rigaud[2]

[1]E.N.T. Department and [2]Biochemistry and Molecular Biology, Limoges University Hospital Center 2, Avenue Martin Luther-King, 87000 Limoges, France

Setup of animal models of cancer is indispensable for pre-clinic therapeutic assays. We developed a new animal model of squamous cell carcinoma from a tumor induced by 4-nitroquinoline-1-oxyde (4-NQO) by successive tumoral grafts in Sprague Dawley immunocompetent rats aged 21 days. Using the same protocol, a model of mandible osteosarcoma was obtained by grafting osteosarcoma tumors. These tumors were characterized by pathological, immunohistochemical analysis and imaging using positron emission tomography coupled with computed tomography (PET/CT). They presented similar characteristics as human ones. We report a therapeutic test using *bevacizumab* (Avastin®) on osteosarcoma rat models. *Bevacizumab* showed a statistically significant effect ($p<0.001$) on development of osteosarcoma.

We propose a protocol to produce animal models of cancer in immunocompetent rats.

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TPA SERUM LEVELS IN PATIENTS WITH SUSPICIOUS SIGNS OF LUNG CANCER: COMPARISON WITH OTHER TUMOR MARKERS USED IN THIS MALIGNANCY (CEA, SCC, NSE, CYFRA 21-1, CA 125, CA 19.9, CA 15.3 AND TAG-72.3)

J.M. Auge[1], R. Molina[1], J.M. Escudero[1], X. Filella[1], R. Marrades[2] and N. Vinolas[3]

Departments of [1]Biochemistry, [2]Pneumology and Medical [3]Oncology Hospital Clinic, Barcelona, Spain

Tumor marker serum levels were prospectively studied in 267 patients with suspicious signs of lung cancer, being the final diagnosis, in 58 patients with no malignancy (15 infectious, 43 non infectious diseases), 28 patients with malignancies excluding lung cancer and lung cancer in the remaining 181 patients (146 NSCLC, 35 SCLC). Slightly high abnormal serum levels were found in CEA 8,6%, CA 19.9 and CA 125 in 22.4%, NSE 0%, CYFRA 3.4%, TAG 72.3 12.1%, SCC 1.7%, CA 15.3 in 3.4% and TPA in 13.7% of the patients with benign diseases. Significantly higher concentrations of TPA, CA 19.9 and CYFRA 21.1 were found in patients with infectious diseases than in other benign pathologies. ($p=0.002$, 0.01 and 0.02, respectively).

Tumor marker sensitivity was related to cancer histology and tumor extension. Significantly higher NSE serum levels and sensitivity was found in SCLC than in NSCLC ($p=0.001$). SCC, CEA, CA 15.3 and CA 125 were also related to the histological type, with significantly higher values in patients with NSCLC ($p<0.02$ in all of them). CYFRA and TPA, show a high concordance of results in patients with lung cancer, in both NSCLC and SCLC (85.5% and 91.4%, respectively). The best combination of tumor markers was CEA, CA 15.3 and one cytokeratin in adenocarcinomas and CEA, SCC and one cytokeratin in squamous tumors.

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ANTICANCER STRATEGIES TARGETING TELOMERASE AND TELOMERES

C. Autexier[1,2], M.E. Brault[1], P. Englebienne[3], J. Fakhoury[2], R. Kieltyka[3], N. Moitessier[3] and H. Sleiman[3]

Departments of [1]Anatomy and Cell Biology, [2]Medicine, [3]Chemistry, McGill University, Montreal QC, Canada

Infinite replicative potential requires telomere maintenance, which, in most eukaryotic organisms, is mediated by telomerase. Telomerase is a ribonucleoprotein that consists of a catalytic reverse transcriptase TERT, associated proteins and an integral RNA subunit (hTR in humans) that carries the template to generate telomeres *de novo*. Eighty-five percent of tumor cells maintain telomeres through an active telomerase complex. Telomere dysfunction can lead to senescence or apoptosis and impair the continued growth of immortal cancer cell lines. Thus anticancer strategies which target telomerase and telomeres are actively investigated.

An alternative to telomerase inhibition-based therapy consists of targeting the integrity of telomeres rather than telomerase activity. One of theses approaches has been validated by us and others and consists of destabilizing telomeres with a mutant human telomerase RNA (hTR) template that dictates the synthesis of mutant telomere sequences. Cell lines expressing a mutant hTR (MuAhTR) exhibit increased sensitivity to chemotherapeutic drugs such as DNA damaging or cytotoxic agents, manifested by decreased cell proliferation. Consistent with the hypothesis that MuAhTR expression engages a DNA damage response, we found increased 53BP1 and ATM-P foci at the telomeres by immunofluorescence in MuAhTR expressing cells compared to cells not expressing MuAhTR. We are currently analyzing the mechanisms implicated in the increased sensitivity of these cells to drugs and the requirement for p53 activation. For example, treatment with doxorubicin leads to altered cell cycle profile and apoptosis in one cell line. We are also validating the specificity of MuAhTR-mediated telomere destabilization in telomerase-positive cells. Since telomerase is absent or weakly active in primary cells, we hypothesize

that expression of a mutant hTR should minimally affect the proliferation of primary cells.

Telomere integrity can also be compromised *via* telomere disruption by G-quadruplex ligands. Such ligands typically stabilize the G-quadruplex structures that can form at telomeres. Such structures are poor substrates for telomerase and several G-quadruplex ligands have been reported to mediate antiproliferative responses in cancer cells. Molecules with distinct structural features, unattainable using conventional covalent synthesis, can be generated using supramolecular self-assembly, and would be particularly useful for targeting higher-order biological motifs such as the G-quadruplex. The generation of such a molecule, and evidence that it is an efficient G-quadruplex binder and telomerase inhibitor will be discussed.

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EURYCOMANOL FROM *EURYCOMA LONGIFOLIA* JACK EXHIBITS ANTIPROLIFERATIVE ACTIVITY ON HUMAN BREAST CANCER CELLS MCF-7 *VIA* APOPTOSIS INDUCTION

Tee Thiam Tsui[1], Cheah Yew Hoong[1], Meenakshii Nallappan[2] and Hawariah Lope Pihie Azimahtol[1]

[1]School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor;
[2]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

The present study investigated the antiproliferative effect, mode of cell death and the mechanism of action of eurycomanol, a quassinoid from the root of *Eurycoma longifolia* Jack on the human breast cancer cell line MCF-7. Eurycomanol exhibites cytotoxic activity towards MCF-7 (IC_{50}=15.23±0.66 μg/ml) and is less sensitive against a normal breast cell line MCF-10A (IC_{50}=66.31±0.47 μg/ml). Based on the IC_{50} values obtained, eurycomanol displayed a certain extent of cytoselectivity towards normal breast cells. The antiproliferative activity of eurycomanol was due to apoptosis induced in MCF-7 cells and not necrosis. This was demonstrated by the Hoechst 33258 nuclear staining assay, Tdt-mediated dUTP nick end labeling assay (TUNEL), and double staining (mixture of Hoechst 33258 and propidium iodide). The apoptotic index was found to increase from 0% at 0 h, to ~50% by 24 h and then to >75% after 72 h. The eurycomanol-treated MCF-7 cells also showed typical apoptotic morphology such as DNA fragmentation, cell shrinkage, and nuclear condensation. The apoptosis triggered by eurycomanol in MCF-7 cells was associated with the down-regulation of the antiapoptotic BCL-2 protein expression, but not with BAX and p53. BCL-2 protein expression levels decreased 2 h after treatment with eurycomanol, and remained lower than controls throughout the experiment, resulting in a shift in the BAX to BCL-2 ratio which eventually induced disruption of the mitochondrial membrane potential thus favouring apoptosis. Next, the processing of the 35 kDa executioner procaspase-7 was detected, but initiator procaspase-9 was found not to play a role in caspase-7 activation in MCF-7 cells. Active caspase-7 then cleaved and inactivated poly (ADP-ribose) polymerase (PARP-1), resulting in nuclear fragmentation. These results, therefore, suggest eurycomanol exerts antiproliferative effects on MCF-7 cells by inducing apoptosis through the down-regulation of BCL-2 protein levels, increased BAX to BCL-2 ratio, activation of caspase-7 and inactivation of PARP-1, without the involvement of p53 and caspase-9.

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NANOTECHNOLOGY APPLICATIONS IN siRNA AND ANTISENSE DELIVERY TO SILENCE MDR1 AND EGFR IN RESISTANT BREAST CANCER CELLS

E. Azizi[1,2,5], N. Ashrafi-Shahmirzadi[1], A. Jahangiri[1], Sh. Fouladdel[1,2], F. Talaee[1,3], A.R. Nomani[1,3], T. Gazori[1,3], F. Atyabi[2,3], E. Haririan[3], R. Dinarvand[2,3], A. Shafiee[4], L. Bazargan[1,6] and M. Shahbazi[7]

[1]Molecular Research Lab., Department of Pharmacology and Toxicology, [2]Medical Nanotechnology Research Center, [3]Department of Pharmaceutics, [4]Department of Medicinal Chemistry, Faculty of Pharmacy, [5]Department of Medical Biotechnology, Faculty of Advanced Medical Technologies, Tehran University of Medical Sciences (TUMS), [6]Department of Biochemistry, IBB, University of Tehran, Tehran;
[7]Cellular and Molecular Research Center, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

Drug resistance has been an important obstacle in cancer chemotherapy for many years. Advances in nanotechnology applications for gene and drug delivery open new hopes to overcome the old problem of drug resistance in cancer cells. For many years, chemical and biological compounds have been investigated to reverse mdr1 over-expression that is the most important cause of failure of chemotherapy in different cancers. There is a high homology between the P-gp, encoded by mdr1 gene, and the cytochrome p450 3A4 (CYP 3A4), that is essential for metabolism of certain endogenous and exogenous compounds. In addition, over-expression of certain genes such as EGFR, a proto-oncogene that induce cell proliferation, in cancer cells significantly decreases the efficiency of anticancer drugs. Nanotechnology has employed tiny structures including liposomes, polymers or dendrimers at nanoscale to combat cancer cell proliferation. Recently many research centers have jointly investigated applications of

nanoparticles loaded with siRNA or antisense with or without anticancer drugs to enhance therapeutic efficiency of chemotherapy in mdr1 and/or EGFR over-expressing cancer cells. Several new dihydropyridines as P-gp reversal compounds were synthesized and evaluated *in vitro* on human breast cancer T47D cells resistant to Tamoxifen and cross resistant to Doxorubicin. Two of these compounds showed partial P-gp reversal activity in human breast cancer T47D cells using various methods including MTT assay, Rh123 accumulation/efflux assay by flow cytometry, RT-PCR, and immunocytochemistry. Also, new drug delivery systems, were prepared and evaluated using nanoparticles with different structures containing siRNA and antisense to down-regulate the mdr1 and/or EGFR mRNA in the same resistant T47D cells. We used the MTT assay, RT-PCR and flow cytometric analysis of cell cycle and apoptosis induction to assess the response of parent and resistant T47D cells with or without transfection with siRNA or antisense to Doxorubicin. The results of RT-PCR studies indicated significant reduction (up to 90%) in both mdr1 and EGFR mRNA in cancer cells. The effect of Doxorubicin has also increased significantly (up to 35%) in the MTT assay although at less extent to mRNA reduction. Flow cytometric analysis showed significant S phase arrest by DOX in mdr1 silenced *versus* G_2/M arrest by DOX alone and different levels of apoptosis *versus* necrosis in treated resistant cells compare to RPMI control. In conclusion, the new gene and drug delivery systems using nanotechnology can improve cancer chemotherapy at a great extent.

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EFFECTIVENESS OF NEOADJUVANT CHEMOTHERAPY IN JAPANESE PATIENTS WITH COLORECTAL CANCER LIVER METASTASES

H. Baba, T. Beppu, M. Watanabe, H. Komori, N. Hayashi, E. Toyama, K. Horino and H. Takamori

Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

Background: Patients (Pts) with colorectal liver metastases have a poor prognosis even after curative resection because of high incidence of recurrence in the remnant liver and thus may benefit from preoperative chemotherapy and curative resection. However, neoadjuvant settings of chemotherapy for pts with CRC liver metastases have not yet been established in Japan and need to be assessed. *Methods:* Pts with CRC liver only metastasis initially unresectable were eligible for the single institute, non-randomized phase II trial. Eligible criteria were synchronous or metachronous liver metastases, primally unresectable, organs function preserved, and PS less than 2. Pts received FOLFOX for 6-8 cycles preoperatively. Clinical response, adverse effects, histopathological analysis of tumoral and nontumoral liver, primary lesion and lymph nodes and survival data were assessed. *Results:* Sixty pts with colorectal liver metastasis were admitted to our hospital from May 2005 to March 2008. Of those, 35 pts initially unresectable received FOLFOX and 17 pts turned out to be respectable after a median of 7.5 cycles of FOLFOX and underwent hepatectomy, and 8 pts also had primary tumor resection. There was no severe perioperative complication, and intra-operative increased bleeding. Mean number of tumors was 6.8 and mean tumor size reduced form 5.8 cm to 3.5 cm. Clinical and histopathological response rates were 81.0% and 29.2%, respectively. Complete tumor necrosis of liver metastases was observed in 2 pts and of primary tumor was in 1 pt. Half life time of CEA in responder and nonresponder was 17.9 and 38.5 days, respectively. Two-year progression free and over all survival rates was 50% and 100%, respectively. *Conclusion:* These data suggest that FOLFOX can be safely administered for Japanese as an neoadjuvant setting in CRC liver metastases without increasing perioperative complications. Moreover, high response, respectability, and superior survival data were obtained with this preoperative chemotherapy and keratectomy, suggesting that a future nationwide clinical trial can be warranted.

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MELANOMA *VS.* NEVI PROBLEM IN THE EYES OF THE TRANSCUTANEOUS ELECTRODYNAMIC IMAGING

Yurii F. Babich, Eduard A. Bakai and Maya A. Nuzhdina

Centre of Biomedical Electroengineering, Kiev, Ukraine

Purpose: Further development of early diagnostic criteria of malignancy. *Materials and Methods:* Our approach was based on analysis of initial and hypoxia-induced electrical dynamics, which reflects relative metabolic differences between the tumour and its microenvironment at the background of the adjacent tissues. We have developed a new functional imaging modality of broad application – transcutaneous electrodynamic introscopy (TEI), which enables *in vivo* non-invasive 3-4D visualization (extremely low-intensive electromagnetic fields are used) of the integral spatial electrobiochemical dynamics in normal conditions and pathology. Adequate resolution (less than 1 mm) had already enabled us firstly (20 years ago) to non-invasively read and monitor the skin electrical landscape (SEL) and discover a new class of initial and induced spatial and temporal phenomenological features (reflecting *in vivo* deep processes of tissue metabolism and intercellular signalling), which may be used as novel diagnostics signs, as for real-time assessment of individual reactions and thus for purposes of targeted/controlled therapy. Currently, we are using a portable experimental TEI setup designed for investigation of superficial tissues, specifically for chosen

model objects, *i.e.* directly visible ones: cutaneous melanoma and nevi. This TEI setup enables the tissue (scan-area 32×64 mm) to be scaned simultaneous in six spectral electrical bioimpedance/potential parameters of the tissue at 1 kHz – 1 MHz-band, making thus possible tissue characterization at tissue, cellular and sub-cellular levels. Fifteen healthy volunteers, thirteen nevi and seven malignant melanomas were investigated. The hypoxic test, *e.g.* simple breath-holding for 20-60 s, was used as contrast factors (various mild physicochemical factors and that of X-ray therapy were studied before). *Results:* No abnormal reactions to the hypoxia test were registered in healthy subjects nor in most of those with nevi. Noticeable hypoxia induced SEL dynamics were registered in all melanomas. Distinct SEL local changes appeared one by one around the pigmented zones, thus making visible the tumours' true microenvironment. The hypoxia test also revealed prospective metastasis, *i.e.* remote areas of supposedly chronic or transient hypoxia with characteristic relaxation times (specifically in comparison with those of the tumour microenvironment). A coherent zone of abnormal mitochondrial electropotential was also detected. The zone crossed the area of supposed metastasis and had an epicentre inside the melanoma. Under the test circumstances, the zone was of less variable character. It was also possible to trace phase differences between intercellular media, cellular membranes and sub-cellular responses to the tests. Suspicious SEL changes were also registered in 3 out of 13 nevi, *e.g.* sound spatially directed dynamics taking its start from the nevi boundary. Subsequent histological analysis was effective enough to confirm only the TEI-revealed heterogeneities of the primary melanomas. *Conclusion:* TEI demonstrates an intriguing ability to reveal *in vivo* a unique set of basically important anatomic and particularly functional features which may be used both for: (i) earlier tumour and metastasis diagnosis and, (ii) for targeted therapy (providing an effective biofeedback for real-time assessment of individual sensitivity to therapeutic factors). Any proposition as to joint research particularly that aiming to identify the TEI findings (*e.g.* with the aid of PET, sMRI, *in vivo* confocal laser imaging techniques) would be highly welcomed.

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MITOCHONDRIA AND CANCER: PROSPECTS FOR NOVEL THERAPEUTIC TARGETS

Gyorgy Baffy

Harvard Medical School and VA Boston Healthcare System, Boston, MA, USA

Acquisition of mitochondria was a defining step in the evolution of eukaryotic cells. Mitochondria regulate pivotal cellular functions such as metabolism, bioenergetics, and programmed cell death. Along with this evolutionary milestone, cancer has taken a seemingly inherent position in our life. Selfishly defiant of the rules of multicellular existence, cancer cells may hijack all the robust homeostatic functions of higher-level organisms. By defining aerobic glycolysis, Warburg was the first to link mitochondria and cancer, but the importance of altered energy metabolism in cancer cells has not been fully appreciated for a long time. Recent advances have transformed our concept on the role of mitochondria in cancer cells and invite a review of new approaches for metabolic targeting of cancer cells including aerobic glycolysis and beyond.

In contrast to high-yield oxidative phosphorylation, glycolysis provides high-rate ATP production with a selective advantage to rapidly growing cells that compete for shared resources. Also, excess lactate production may promote interstitial acidification and invasiveness. Moreover, glycolysis may improve redox control *via* the pentose phosphate cycle (a major source of NADPH). Based on these considerations and experimental validation, inhibitors of hexokinase (*e.g.*, 3-bromopyruvate, 2-deoxyglucose, lonidamine) have entered pre-clinical and clinical testing, while additional research is focused on molecular regulators of the metabolic and bioenergetic phenotype in cancer cells.

One of the metabolic regulators is hypoxia-inducible factor (HIF)-1, a transcription factor commonly activated in cancer cells even in the absence of overt hypoxia. By activating pyruvate dehydrogenase (PDH) kinase (PDK), HIF-1 blocks pyruvate entry to the Krebs cycle and limits mitochondrial electron transport. Recent studies indicate that cellular energy metabolism is also regulated by the tumor suppressor p53. By gene-level suppression of glycolysis (TIGAR) and promotion of electron transport (SCO2), p53 shifts ATP production back to the mitochondria. Thus, successful adaptation of energy metabolism in cancer cells is linked to purposeful restraint of mitochondrial respiration, while tumor suppression may rely on an opposite action.

To fully understand the impact of 'mitochondrial neglect' in cancer cells, it is also important to recall that mitochondrial electron transport is a major source of intracellular reactive oxygen species (ROS) and cancer cells often struggle with increased oxidative stress. Thus, decreased mitochondrial ROS output may be another benefit of aerobic glycolysis. This is well illustrated by the PDK inhibitor dichloroacetate (DCA), which enforces mitochondrial flux of pyruvate, inducing apoptosis of cancer cells *via* increased ROS production and caspase activation.

Our recent observations on the role of mitochondrial uncoupling protein-2 (UCP2) in cancer cells provide a new aspect to the metabolic adaptation of cancer cells. UCP2 is a mitochondrial anion carrier protein located in the inner membrane along with the respiratory complexes. By mediating proton leak, UCP2 controls the rate of superoxide production and acts as a potent suppressor of oxidative stress. Cancer cells may exploit this effect and increased UCP2 expression

has indeed been linked to advanced neoplastic changes and chemoresistance. We found that overexpression of UCP2 in colon cancer cells inhibits ROS accumulation and apoptosis induced by cytotoxic treatment. The protective effect was also observed in tumor xenografts. By contrast, UCP2 silencing appears to enhance the efficacy of chemotherapy. Also, UCP2 overexpression interferes with post-translational modification of p53 and preferentially induces the glycolytic phenotype. Accordingly, inhibition of UCP2 modulates energy metabolism in cancer cells and this approach may be considered in combination treatment of chemoresistance.

In summary, recent advances confirm that oncogenic and metabolic regulatory pathways are strongly interconnected. New therapeutic strategies may become available through better understanding of metabolic and energetic adaptation of cancer cells, with a particular attention to the role of mitochondria in this process.

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IN VITRO MODELING FOR THE EVALUATION OF Lu-177 ANTISENSE RADIOTHERAPY

Ethan R. Balkin[1], Fang Jia[2,3], William H. Miller[3,4] and Michael R. Lewis[2,3,4]

[1]Veterinary Pathobiology, University of Missouri-Columbia, Columbia, MO, USA;
[2]Veterinary Medicine and Surgery, University of Missouri-Columbia, Columbia, MO, USA;
[3]Research, Harry S Truman Memorial Veterans' Hospital, Columbia, MO, USA;
[4]Nuclear Science and Engineering Institute, University of Missouri-Columbia, Columbia, MO, USA;
[5]Missouri University Research Reactor, University of Missouri-Columbia, Columbia, MO, USA

The protein product of the *B-cell lymphoma/leukemia-2* (*bcl-2*) proto-oncogene in non-Hodgkin's lymphoma (NHL) is a dominant inhibitor of apoptosis. In aggressive lymphoma, large cohort studies have shown that the overexpression of the *bcl-2* gene correlates strongly with resistance to radiation and chemotherapy, increased survival of cancer cells, high relapse rate, and poor cause free survival rate or disease free interval. Thus, patients who are found to overexpress *bcl-2* might respond better to alternative treatments like targeted immunotherapy, radioimmunotherapy, or antisense therapy, all of which act through mechanisms that down-regulate *bcl-2*.

Human NHL also expresses Type 2 Somatostatin Receptors (SSTR2) in approximately 87% of cases. Previous work indicates that ^{177}Lu-DOTA-Tyr^3-octreotate provides effective, selectively targeted radiotherapy in human SSTR expressing tumors. Furthermore, this peptide is an attractive vehicle for delivery of other tumor-targeting agents, such as those designed to act against *bcl-2*.

In vitro uptake, efflux, proliferation and viability assays are designed to assess the bcl-2 mRNA targeting of a ^{177}Lu-labeled *bcl-2* antisense peptide nucleic acid (PNA)-Tyr^3-octreotate conjugate against the human NHL cell line Mec-1 in suspension culture. *In vitro* cell uptake and efflux was evaluated by assaying the ^{177}Lu-labeled anti-*bcl-2* PNA-peptide conjugate. The percent uptake of the total radioactivity was found to increase from 1.1±0.5% at 1 min to 2.5±1.1% at 4 hr. The percent retention *versus* time was found to decrease from 71.0±8.5% of the cell-associated radioactivity at 1 min to 29.5±2.6% at 4 hr.

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ARSENIC INDUCED APOPTOSIS IN THE LYMPHOCYTES OF INDIVIDUALS EXPOSED TO ARSENIC THROUGH DRINKING WATER IN WEST BENGAL, INDIA

Apurba K. Bandyopadhyay, Nilanjana Banerjee and Ashok K. Giri

Molecuar and Human Genetics Division, Indian Institute of Chemical Biology, Kolkata- 700 032, India

In West Bengal, India, more than 6 million people in nine districts are exposed to arsenic through drinking water. It is regarded as the greatest arsenic calamity in the world. Arsenic is a well-documented human carcinogen, which does not induce cancer in any other animal model. Interestingly, at lower concentrations, arsenic is known to induce apoptosis in various cancer cell lines *in vitro*. We have studied apoptosis in human peripheral blood mononuclear cells (PBMC) of arsenic exposed skin lesion individuals by annexin V-FITC staining and compared it with that in unexposed individuals. The percentage of apoptotic cells in individuals with skin lesions was significantly higher ($p<0.001$) in comparison to unexposed individuals. In the exposed individuals with skin lesions, there were elevated levels of intracellular reactive oxygen species (ROS), mitochondrial membrane permeability and increased cytochrome *c* release, leading to increased downstream caspase activity. Arsenic-induced DNA damage was confirmed by DNA ladder formation and confocal microscopy. These findings suggest that chronic arsenic exposure causes cell cycle arrest at the G1-phase. Arsenic causes significant DNA damage, and DNA damage is known to trigger apoptosis. Results of DNA laddering and confocal microscopy confirm that chronic arsenic exposure leads to DNA damage and thus might lead to enhanced apoptosis in the exposed group. Cell apoptosis is a normal physiological process by which correct functional cellular populations are maintained, by removal of cells with abnormal genetic information. Arsenic, like some other metals, plays a dual role in that they have apoptotic effects on the cells but also

contribute to cell transformation and carcinogenesis. The exact mechanism of how this switching occurs is not known yet. It may so happen that increased apoptosis of certain immune cells, such as NK cells and cytotoxic T-cells, could impair the immune surveillance against cancer in various organs and thus explain the increased risk of cancer. Another possible explanation is that metals under certain conditions cause greater apoptosis, but it is not known whether this apoptosis induced by the metal is a perfect process or an imperfect process. An imperfect apoptotic process might result in the escape of cells that would be potentially carcinogenic. This may be true in the case of arsenic-induced apoptosis also, which ultimately leads to the various types of cancer. Further research is required to determine exactly whether increased apoptosis of immune cells or defective apoptosis is responsible for arsenic-induced cancer of skin and other internal organs.

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THE BANERJI PROTOCOLS: THE REGRESSION OF MALIGNANT TUMOURS BY A NON-INVASIVE AND NON-TOXIC ORAL MEDICAL APPROACH

Prasanta Banerji and Pratip Banerji

PBH Research Foundation, 10/3/1 Elgin Road, Kolkata-700020, India

Background: Although there is significant improvement of treatment outcomes of most malignant tumours by conventional therapy, in many neoplasms, particularly oesophageal, brain and lung tumours, there is still a persistent grim outlook of conventional therapy results. To obviate this long lasting problem, we have treated many patients belonging to this group with ultra diluted medicines (under Government-regulated standard pharmacopeias in different countries) which are commonly used in homeopathic practice. In the course of our relationship with various scientific bodies, we have previously presented before the NCI, USA, a Best Case Series on Cancer, which was unanimously accepted by the Advisory Panel. The outcome of our treatment with these ultra-diluted medicines is described here. *Materials and Methods:* During the past year, we have treated 1132 malignant tumour cases belonging to these categories, in our busy clinic located in Kolkata, India: 689 cases of brain tumours were treated with Ruta 6 and Calcarea Phosphorica 3x; 367 lung cancer cases were treated with Kali Carbonicum 200c and 76 oesophageal carcinoma cases were treated with *Condurango* 30c. All available clinical, histological and radiological records for diagnosis and follow-ups were analyzed. *Results:* Complete regression was noted in 32% cases of brain tumours, 22% lung cancer cases and 28% oesophageal carcinoma cases and we are aiming to discontinue their treatment. *Conclusion:* At present in our clinics in Kolkata, India, we use these ultra-diluted medicines as the sole specific therapy in cases of malignant tumors, with encouraging results. From our results, it is apparent that these medicines can also be given as an adjunct therapy in other countries in all such malignant cases where conventional therapy fails. We will present here some cases of lung, brain and oesophageal malignancies treated solely by the Banerji Protocols to show the remarkable efficiency of these ultra-diluted medicines.

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FUNCTIONAL MODULATION OF DENDRITIC CELLS AND THEIR PRECURSORS IN THE TUMOR MICROENVIRONMENT

J.A.M. Barbuto, P.C. Bergami-Santos, A.P.S. Azevedo, R.B. Baleeiro and M. Tregia

Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Av. Prof. Lineu Prestes 1730, 05508-000 - Sao Paulo, SP, Brazil

Dendritic cells (DC) have a unique role in the establishment of immune responses. Their ability to recognize the "status" of the environment and translate it into specific activation signals for clonally selected lymphocytes allows the immune system to react appropriately to microbial challenges at the same time as it tolerates innocuous antigens. Accordingly, the decision to tolerate or to react against tumor cells should also depend on the DC reaction to the tumor microenvironment. Since clinically significant tumors frequently generate potentially activating signals for DC, which, in turn, should trigger an immune reaction against tumors, the development of progressive cancer in immunocompetent individuals could be a sign of an altered DC activation. Indeed, we show here that the antigen-presenting cells' phenotype (expression of HLA-DR, S100, CD1a, CD11c, CD14, CD68, CD80, CD86 and CD83) within tumors was altered, with an imbalance between macrophages, immature and mature DC, a phenomenon that could be correlated to the pattern of cytokine secretion (IL4 and TNF-alpha) within the tumor environment. Furthermore, the analysis of the differentiation of cancer patients' monocytes into DC suggested that this phenomenon was not restricted to the tumor site. Monocyte-derived DC from cancer patients also presented an altered membrane phenotype and responded poorly to environmental challenges found in cancer, as low local pH, jaundice or the presence of tumor cells. The mechanisms responsible for these phenomena still need to be characterized, but preliminary data suggest that they may depend on low levels of phosphorylated STAT-1 in cancer patients' DC.

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NOSCAPINE INHIBITS PROSTATE CANCER

Israel Barken

Prostate Cancer Research and Education Foundation, La Mesa, CA, USA

Background: Noscapine, a non-toxic, alkaloid and common constituent of cough medicine, stabilizes tubulin. It inhibits the growth of several human and murine neoplasms, with no significant toxicity. Its effect on prostate cancer has not been evaluated. *Materials and Methods:* Noscapine was administered orally (300 mg/Kg per day) for 56 days to PC3 humans prostate cancer-bearing immunodeficient mice (n=10). Immunodeficient control mice (n=10) received only diluent in an identical regimen. *Results:* Mean total tumour weight was 0.42±0.23 g and 0.97±0.31 g ($p<0.001$) in the noscapine treated group and the control group, respectively, without evidence of toxicity. Metastases occurred less frequently in the treatment than the control group (30% *vs*. 90%; $p<0.05$). *Conclusion:* Oral administration of noscapine limited tumour growth and lymphatic metastasis of PC3 human prostate cancer in this mouse model, supporting its therapeutic potential as a nontoxic and easily administered treatment for metastatic cancer.

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EXPRESSION PATTERN OF THE UBIQUITIN C-TERMINAL HYDROLASE IN RAT COLON AND THE HUMAN COLON CARCINOMA CELL LINE HT-29

Torsten Bartz, Juergen Bartel, Anja Soete, Elzbieta Charkiewicz and Antonios Kyriakopoulos

Helmholtz Centre Berlin for Materials and Energy, Department" Molecular Trace Element Research in the Life Sciences", Glienicker Str. 100, D-14109 Berlin, Germany

The ubiquitin proteasome system (UPS) contains the components for one of the major mechanisms for the regulation of protein levels in eukaryotic cells. Simplified, protein designated for degradation forms a complex with chains from ubiquitin or polyubiquitin and the complex is transferred to the 26S proteasome under contribution of several enzymes, where – after removal of the ubiquitin – the targeted protein is cleaved. A member of the family of the deubiquitinating enzymes (ubiquitin c-terminal hydrolase isozyme L3; UCH-L3), is suggested to have anti-apoptotic properties, and autoantibodies were detected in sera from colon cancer patients.

We have recently demonstrated altered expression profiles of UCH-L3 in hepatocytes grown under conditions of moderate, non-toxic oxidative stress and in rat livers exposed to oxidative stress by selenium deficiency. Here we investigated the expression pattern of this enzyme in colons obtained from rats with varying selenium status and the colon cancer cell line HT-29 cultured in the presence of various metal ions.

Therefore cells were incubated individually with high concentrations of molybdenum, manganese, selenium, cadmium, or other metals for 72 hour each. As determined by tetrazolium assay the applied concentrations lead to minor cell proliferation. Harvested cells and colon tissues from selenium adequate or selenium deficient rats were homogenised and UCH-L3 was detected by western blotting and immunochemistry.

By using beta-actine as an internal standard our results showed that the expression of UCH-L3 was elevated in HT-29 cell line and selenium deficient colon tissue about 1.5-fold compared to rats fed with an adequate selenium diet. Admittedly this increase was much lower then that caused by the incubation with high concentrations of manganese (4-fold). A possible explanation might be the involvement of manganese in the protection against reactive oxygen species as a compound in the superoxide-dismutase.

Although further investigations will be needed to understand the involvement of UCH-L3 in selenium's cancer protective effects, we propose that selenium deficiency causes at least two changes in the cell that can lead to cancer in the end: Primary a reduction in the protection against specific oxidants by the loss of selenoproteins. And secondly the induction of UCH-L3 would prevent a removal of oxidatively damaged cells by apoptosis.

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ANTI-ANGIOGENIC ISOFORMS OF VEGF – A KEY TO ANTI-ANGIOGENIC THERAPY

D.O. Bates and S.J. Harper

Microvascular Research Laboratories, Department of Physiology and Pharmacology, Bristol Heart Institute, Southwell Street, Bristol BS2 8EJ, UK

VEGF is generated from differential splicing of 8 exons, with exons 6 and 7 being differentially spliced to generate isoforms with altered heparin and neuropilin binding affinities. An additional alternate splice site exists in exon 8, which results in a more distal splice site selection in this final exon. This results in a sister family of isoforms termed $VEGF_{xxx}b$, which differ only in their terminal 6 amino acids from conventional VEGF isoforms. Using antibodies generated to the unique c-terminal protein sequence, we have found that the $VEGF_{xxx}b$ isoforms are widely expressed at the protein level in normal human tissues. In fact in most tissues studied, they appear to be the predominant isoforms with tissues varying from 96% of total VEGF being $VEGF_{xxx}b$ (colonic mucosa), to 40% (bladder). In contrast, the placenta, an angiogenic tissue does

not express much $VEGF_{xxx}b$ (1.5% of total VEGF). These isoforms are downregulated in cancers and other pathologies associated with abnormal angiogenesis (cancer, diabetic retinopathy, retinal vein occlusion, Denys Drash syndrome, and pre-eclampsia). Using human recombinant $VEGF_{165}b$, we have shown that this terminal splice site selection causes these isoforms to lose angiogenic activity, and to inhibit $VEGF_{165}b$ mediated angiogenesis in the rabbit corneal eyepocket, the rat mesentery and the mouse retina and choroid. Furthermore these isoforms, when over-expressed by tumour cells inhibit growth of colorectal carcinoma, renal cell carcinoma, prostate cancer, malignant melanoma and Ewing's sarcoma. Furthermore, local administration of recombinant $VEGF_{165}b$ inhibits the growth of colorectal carcinoma cells *in vivo*. Recombinant $VEGF_{121}b$ also appears to inhibit migration of endothelial cells stimulated by $VEGF_{165}$. Thus these isoforms form a family of anti-angiogenic VEGFs. We have identified a number of cytokines that alter the regulation of the splice site, such that cells can switch from the anti-angiogenic $VEGF_{xxx}b$ isoforms to the pro-angiogenic $VEGF_{xxx}$ isoforms, including IGF1, TNF-α and PDGF-AB. The reverse switch can be induced by treatment with TGF-β1. These isoforms are recognize by most VEGF antibodies, including bevacizumab, and over-expression of $VEGF_{165}b$ inhibits the anti-angiogenic action of bevacizumab in mouse models of human cancers. In summary, C terminal distal splicing is a key component of VEGF biology, overlooked by the vast majority of publications in the field, and these findings require a radical revision of our understanding of VEGF biology in normal human physiology.

Harper SJ and Bates DO: VEGF splicing – the key to anti-angiogenic therapy? Nature Cancer Reviews. 2008. In press.

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MOLECULAR PATHOLOGY OF DUCTAL BREAST CANCER

Anna Batistatou

Department of Pathology, University of Ioannina, Medical School, Ioannina, Greece

Based on the multistep model of breast cancer progression sequential steps in the development of invasive ductal carcinoma (IDC) are usual ductal hyperplasia, atypical ductal hyperplasia, ductal carcinoma *in situ* (DCIS) and IDC. Although this theory of "linear progression" is appealing, there are no consistent molecular data to support the correlation between premalignant, preinvasive and invasive ductal carcinoma. The alternative theory of breast cancer development is that of "parallel disease". According to this theory, DCIS is classified as low- and high-grade and is believed to progress in low- and high grade IDC respectively, independently, in parallel fashion. Today, despite intensive molecular research, we are not able to predict with certainty the risk of DCIS progression to IDC or recurrence. Besides standard histopathological parameters, such as nuclear grade and necrosis, biological markers that appear promising are: the status of steroid receptors (ER, PgR), markers of proliferation and regulators of cell cycle such as MIB-1, bcl-2, p53, p21, cyclin D1 and cyclin A, adhesion molecules, such as E-cadherin, matrix metalloproteinases, regulators of angiogenesis (growth factors and their receptors), such as VEGF-A, VEFG-C, and VEGFR-1 (Flt-1), VEGFR-2 (Flk-1) and VEGF-R3 (Flt-4).

The great progress in understanding invasive breast cancer has come during the last decade from gene expression profiling studies, which have established a molecular classification, with relevance to patient outcomes. So, it has become apparent that the morphological heterogeneity of invasive breast cancer, which has been appreciated by pathologists for the last 4-5 decades, is reflected at the transcriptomic level. The molecular classification has been developed based mainly on cases of IDC, and distinguishes five main groups: luminal A and luminal B (both ER+), basal-like, HER2+ (ERBB2) and normal breast-like. The last three subtypes are usually ER–. Furthermore, prognostic gene sets have been developed, including a 70-gene prognosis profile and a 21-gene recurrence score. Pathologists follow closely the molecular progress in IDC classification and have incorporated much of this new information in every-day pathology practice. Today, besides ER and PgR, characterization of HER2 expression has become an integral part of the work-up of a breast cancer patient in the Pathology laboratory and of the corresponding pathology report. This practice has led to identification of new entities and use of novel terms, such as triple-negative cancer, which encompasses a heterogeneous group of neoplasms with distinctive, but quite variable, pathologic and clinical features.

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SURGICAL APPROACH OF BREAST CANCER: AN UPDATE

Ch. Batsis

Medical School, University of Ioannina, Ioannina, Greece

Since 1894 when William Halsted introduced the radical mastectomy as the definitive surgical procedure for the

treatment of breast cancer, remarkable progress has been made in the local treatment of this disease in the last 100years, reflecting changes in our understanding of the biology of breast cancer and improvements in diagnostic and therapeutic modalities. The breast conserving surgery (BCS) and radiotherapy has become more common, as surgeons have become convinced of the efficacy of this treatment through data from large prospective randomized trials. Veronesi, Fisher and others have shown the surgical rates with mastectomy and BCS plus radiotherapy to be equivalent. Because of the complexity of the disease process, the availability of multiple treatment options and the need to individualize each woman's care, a multidisciplinary approach is essential to optimize the treatment.

Discussion of the local treatment of breast cancer should be divided into treatment of invasive breast cancer and treatment of ductal carcinoma *in situ* (DCIS). Invasive breast cancer and DCIS are clinicopathologic entities that require different treatments. Furthermore, treatment of invasive cancer must be divided into early breast cancer (stage I-II) and advanced breast cancer (stage III-IV). Most women with stage I-II breast cancers are good candidates for BCS and radiation but there are certa*in situ*ations where the mastectomy is probably the best choice of therapy. The standard of care in treatment of the axilla for patients with invasive breast cancer is currently a level I and II axillary dissection. Sentinel node biopsy of the axilla would decrease the risk of side-effects from dissection, while still accurately staging the axillary nodes in appropriate clinical situations. The treatment of stage III breast cancer should be decided after multidisciplinary consultation, with a combination of neoadjuvant chemotherapy or initial hormonal treatment, making surgery feasible some weeks or months later. The local treatment of stage IV is palliative and not life saving.

The treatment of DCIS consists of local treatment alone. Most women are good candidates for BCS alone or BCS and radiotherapy but there are some contraindications where simple mastectomy with or without reconstruction is preferable. Lobular carcinoma *in situ* (LCIS) is a rare pathologic entity poorly understood by patients and this is considered rather as a marker that places a woman at inscreased risk for the subsequent development of invasive cancer. The treatment options include close follow-up or bilateral prophylactic mastectomy with or without reconstruction.

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THE ROLE OF XRCC4 IN CARCINOGENESIS AND ANTICANCER DRUG DISCOVERY

Da-Tian Bau

Departments of Terry Fox Cancer Research Lab, China Medical University Hospital, Taichung, Taiwan, ROC

In the past decades, the incidence of cancer continues to rise rapidly all over the world and is always an important threat to public health. It is believed that cancer results from a series of genetic alterations leading to progressive disorder of the normal mechanisms controlling cell proliferation, differentiation, death, and/or genomic stability. The response of the cell to genetic injury and its ability to maintain genomic stability by means of a variety of DNA repair mechanisms are therefore essential in preventing tumor initiation and progression. From the same viewpoint, the relative role of DNA repair as a biomarker for prognosis, predicator of drug and therapy responses, or indeed as target for novel gene therapy (recently patented) and is very promising. Here, we summarize and evaluate associations between the SNPs of *XRCC4*, one of the NHEJ genes, and the susceptibility of multiple types of cancer, and discuss its role in carcinogenesis and application in anticancer drug discovery.

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SENSITIZATION OF TUMOR CELLS FOR ROS - MEDIATED INTERCELLULAR APOPTOSIS INDUCTION: A NOVEL CHANCE TO ESTABLISH SELECTIVE ANTITUMOR MECHANISMS

Georg Bauer

Department of Virology, University of Freiburg, Freiburg, Germany

Transformed cells are subject to the control by intercellular induction of apoptosis, a TGF-beta-triggered, reactive oxygen species (ROS)-mediated control mechanism that selectively removes precancerous cells. The efficiency of intercellular induction of apoptosis, as well as its selectivity with respect to the transformed state of target cells, is driven by extracellular superoxide anions that are generated by transformed cells. Four ROS-based intercellular signaling pathways contribute to intercellular induction of apoptosis. During tumor progression, transformed cells acquire resistance mechanisms against intercellular induction of apoptosis. This resistance is based on active interference of tumor cells with intercellular ROS-mediated signaling through membrane-associated enzymes. Resistance against intercellular ROS-signaling is found in all tumor cell systems tested, including the most frequent and the most aggressive human tumors.

Resistant tumor cells can be sensitized for intercellular ROS signaling and subsequent selective apoptosis induction through several distinct approaches established by our group. Among them are: i) siRNA-mediated knock down of the major interfering enzyme; ii) inhibition of interfering enzymes by defined small molecules; iii) inhibition of interfering enzymes by monoclonal antibodies; iv) generation of singlet oxygen through modulation of the tumor cells ROS signaling chemistry. The latter approach utilizes tumor cell specific ROS

interactions for singlet oxygen generation as a first step, followed by inactivation of the major interfering enzyme and subsequent efficient intercellular ROS signaling, leading to selective tumor cell apoptosis.

Ligand-dependent and independent death receptor activation as well as low-dose gamma irradiation show synergistic interactions with the sensitization of tumor cells and with intercellular ROS signaling. These synergistic effects may be utilized for further optimization of the sensitization process.

As intercellular ROS-signaling is specific and typical for cells with the transformed phenotype and as resistance against ROS signaling seems to represent a regular phenotypic characteristic of tumor cells, sensitization of tumor cells for intercellular ROS signaling should allow to establish novel tumor therapeutic approaches that bear the chance for high selectivity and efficiency.

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REACTIVE OXYGEN AND NITROGEN SPECIES (ROS AND RNS) IN ANTICANCER MECHANISMS

Georg Bauer

Department of Virology, University of Freiburg, Freiburg, Germany

Transformed and tumor cells are characterized by membrane-associated NADPH oxidase and extracellular superoxide anion generation. Extracellular superoxide anions drive the efficiency and selectivity of intercellular induction of apoptosis, an ROS-mediated mechanism that leads to the selective elimination of transformed cells. Intercellular induction of apoptosis is based on four ROS/RNS-based signaling pathways, which may either interact synergistically or in an inhibitory mode. During tumor progression, tumor cells acquire resistance against intercellular ROS signaling through specific interference with intercellular ROS signaling by defined mechanisms.

Our data allow the following conclusions: i) multistep oncogenesis: ROS/RNS-driven intercellular induction of apoptosis might lead to the elimination of premalignant, ROS-sensitive transformed cells during multistep oncogenesis and thus represent a hitherto unrecognized natural control system. The interaction of certain secondary plant products and intercellular ROS signaling leads to the sensitization of ROS-resistant tumor cells (at later stages of oncogenesis) for intercellular induction of apoptosis. ii) Tumor therapy: Apoptosis induction in tumor cells by established chemotherapeutics like taxol or epothilone B depends on functional intercellular ROS signaling. Besides its direct apoptosis-inducing effect, mediated by singlet oxygen, photodynamic therapy leads to sensitization of tumor cells for intercellular ROS signaling. Death receptor activation and intercellular ROS signaling are interconnected and thus amplify apoptosis induction.

The knowledge of the functional role of ROS/RNS during multistep oncogenesis and tumor therapy may be useful to optimize tumor prevention and to improve tumor treatment.

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ANTIOXIDANTS IN MULTISTEP ONCOGENESIS

Anna Burcza and Georg Bauer

Department of Virology, University of Freiburg, Freiburg, Germany

Natural antioxidants from food are well–known for their scavenging activity for reactive oxygen and nitrogen species (ROS and RNS) such as hydroxyl radicals, hydrogen peroxide or peroxynitrite. This scavenging activity may inhibit ROS/RNS-mediated mutagenesis and thus prevent the initiation step of multistep oncogenesis.

Certain flavonoids have been shown to exhibit chemopreventive as well as therapeutic potential through induction of apoptosis in tumor cells (please see the contribution by C. Gerhäuser in this symposium).

Here we report on the potential of flavonoids and other antioxidants to sensitize ROS-resistant tumor cells for intercellular induction of apoptosis, an ROS-driven intercellular signaling system with possible relevance for multistep oncogenesis and tumor therapy. At the first sight, it seems a paradox that antioxidants can trigger and enhance this prooxidative mechanism which is directed against transformed and tumor cells. Our detailed analysis demonstrates a complex network of ROS/RNS/antioxidant interactions. As the first step, antioxidants increase the available nitric oxide (NO) concentration through inhibition of the consumption reaction between hydrogen peroxide and NO. In addition, certain compounds inhibit NO dioxygenase and thus prevent consumption of NO. In a complex series of biochemical reactions, the increased NO concentration finally leads to singlet oxygen generation. Singlet oxgen restores the ability of tumor cells for intercellular ROS signaling. Ongoing ROS signaling first leads to the consumption of the antioxidant which is the prerequisite for subsequent ROS-mediated apoptosis induction. Overall, tumor cells that have escaped ROS-mediated intercellular induction of apoptosis through establishment of specific resistance mechanisms are rendered sensitive for ROS-dependent apoptosis induction through the action of the antioxidant.

These data add an unexpected aspect to the multifaceted picture of antioxidant action during multistep oncogenesis. Our findings may be useful for the understanding of the role of nutritional antioxidants in tumor prevention as well as in tumor therapy.

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CANCER CHEMOPREVENTION WITH MICRONUTRIENTS – DNA-PROTECTIVE EFFECTS IN HUMAN MUCOSAL CELL CULTURES

P. Baumeister, N. Kleinsasser and U. Harréus

Department of Otolaryngology/Head and Neck Surgery, Ludwig Maximilians University Munich, Germany

Objectives: In the past decades, large interventional trials were carried out to test cancer chemopreventive effects of nutrional supplementation with micronutrients such as vitamins or trace elements. Unfortunately, no positive influence on cancer incidence could be shown. On the other hand, epidemiological studies suggest that a diet rich in fruits and vegetables has strong cancer chemopreventive effects. We here present an overview of our studies evaluating DNA protection of different vitamins, several polyphenols and zinc. *Methods:* Miniorgan cultures (MOCs), completely epithelised tissue cubes from fresh biopsies of nasal and oropharyngeal mucosa, were pretreated with micronutrients. DNA damage was then introduced oxidatively or with a metabolically activated carcinogen. DNA protective effects were evaluated using the alkaline single cell microgel electrophoresis (Comet) assay. *Results:* All tested micronutrients show remarkable DNA protective action against oxidative or carcinogen-induced damage in our model. Our results indicate their potential to prevent DNA fragmentation from endogenous and/or xenobiotic sources. The lack of this effect in interventional trails could be due to their metabolism.

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CANCER PATIENTS SUFFERING FROM TUMOR-INDUCED EVASION OF THE ADAPTIVE IMMUNITY BY INCREASED IL-4, IL-6 AND IL-10 MAY BENEFIT FROM ANTICANCER TH2 CYTOKINES ANTAGONISTS

Yechiel Becker

Department of Molecular Virology, Facultty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

Background: human tumors release antigenic glycoproteins that are processed by dendritic cells and presented by HLA class I and HLA class II to $CD4^+$ T-cells in the lymph nodes. $CD4^+$ T-cells that interact with HLA class I polarize into two types of T helper 1 cells: Th1 cells produce Th1 cytokines IL-2, IL-12 and interferon gamma while Th17 cells produce the cytokine IL-17. Th1 cells activate the antitumor cytotoxic $CD8^+$ T-cells. Th17 cells are involved in autoimmunity. In addition to the $CD4^+$ and $CD8^+$ T-cells, members of the adaptive immune system, innate system cells, mast cells, basophils, monocytes and dendritic cells, are regulators of the adaptive immune cells, due to their ability to release large amounts of Th2 cytokines that induce B-cells to switch to IgE synthesis. A new test for simultaneous analysis of Th1, Th17 and Th2 cytokines was developed. Passive immunotherapy with humanized monoclonal antibodies: J. King *et al.* reviewed the use of humanized anti tumor antigens monoclonal antibodies (DOI:10.1093/qjmed.hcn050) for treatment of cancer patients. The monoclonal antibody Herceptin (Trastuzumab) binds to the HER2 receptor on metastatic breast cancer leading to 20% reduction of risk of death, but caused 27% increase in cardiac disfunction. The nuclear splicing mechanism of cytokine genes mRNA and splice variants of the genes: The Th2 cytokine IL-4 was found to inhibit antitumor and cytotoxic $CD8^+$ T-cells. Deletion of intron 2 sequnce from IL-4 mRNA yields an IL-4delta2 antagonist that is able to bind to IL-4 receptor alpha on TH2 cells and B-cells and prevents the effects of IL-4 on the adaptive immune response. *Conclusion:* The splice variants of the inhibitory Th2 cytokine IL-4 and IL-6 have potential for use in anticancer treatment.

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"INCLONALS": IgG ANTIBODIES PRODUCED IN E. COLI IN THE CONTEXT OF TARGETED ANTICANCER THERAPY

Rahely Hakim and Itai Benhar

Department of Molecular Microbiology and Biotechnology, the George S. Wise Faculty of Life Sciences, Ramat Aviv 69978, Israel

Antibodies are among the most powerful tools in biological and biomedical research and are presently the fastest growing category of new bio-pharmaceutics. With regard to anticancer immunotherapy, antibodies can be used "naked" as are most FDA approved ones, or as immunoconjugates to direct cytotoxic moieties to tumor cells. Recombinant forms of small antibody fragments fused to cytotoxic moieties, named recombinant immunotoxins, are also being developed as an additional approach for an antibody-targeted cancer therapy.

For treating solid tumors, tumor penetration, which is inversely proportional to the size of the drug, is currently regarded as the prime factor for its efficacy, while other parameters such as binding affinity and residence time in the body are of lesser importance. This dogma may be challenged by recent study from our lab when immunotoxins that target the tumor-associated antigen ErbB2 were evaluated. We found that, a bivalent antibody-toxin conjugate (200 kDa) was much superior to the corresponding recombinant immunotoxin (scFv-toxin fusion, 66 kDa), in killing ErbB2 expressing tumor cells in culture and as xenografts in nude mice. These results suggest that higher avidity and longer residence time may outweigh tumor penetration. This led us to design

additional models to study whether our previous observation was a peculiarity of the model system we used, or a more general phenomenon.

While looking for an easier and more rapid way to achieve the full-length IgGs that are required to this study, we developed an expression and purification protocol for full-length IgGs in *E. coli*, called "Inclonals". By using this protocol, we can obtain a yield of up to 50 mg pure IgG from 1 liter of shake flask culture and highly purified IgGs that are free of contaminating and partially assembled species. The "Inclonals" we produced equaled the performance of the same IgGs that were produced using conventional mammalian cell culture. Antibodies that are produced in *E. coli* are aglycosylated and hence are not useful for purposes that require effector functions such as ADCC and CDC. However, the large majority of antibodies that can be potentially used for therapy, diagnostics or research purposes are not dependent on Fc glycosylation to be effective (some examples are as virus neutralizing antibodies, antibodies that are used to ferry a cargo to the target cells, or bi-specific antibodies). We believe that our rapid and cost-effective IgG production process and the high quality of the resultant product may make bacterial production of full-length IgG, and IgG-based fusion proteins a viable and attractive option for antibody production.

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CIRCULATING TUMOR CELL DETECTION BY CELLSEARCH SYSTEM DURING BREAST CANCER NEOADJUVANT CHEMOTHERAPY

F.C. Bidard[1], C. Mathiot[1], A. Vincent-Salomon[1], R. Salmon[1], M Marty[2] and J.Y. Pierga[1]

[1]Institut Curie, 26 rue d'Ulm, 75005 Paris;
[2]Hôpital Saint Louis, Av Vellefaux, 75010 Paris, France

Background and Aim: Circulating Tumor Cells (CTCs), detected by the CellSearch system in metastatic breast cancer patients have been reported as a surrogate marker for tumor response and shorter survival. The aim of this study was to determine whether CTC are present in the blood of patients with large operable or locally advanced breast cancer before neoadjuvant chemotherapy (pre-CT) and after neoadjuvant chemotherapy, prior to surgery (post-CT). *Patients and Methods:* 7.5 ml blood samples were obtained on CellSave tubes from patients included in a phase II trial (REMAGUS 02). CTCs were immunomagnetically separated and fluorescently stained by the CellSearch® system. Twenty metastatic breast cancer patients were screened as positive controls. *Results:* From 10/2004 to 7/2006, pre-CT and/or post-CT blood samples were obtained from 118 patients. At least one CTC was detected in 22 out of 97 patients with pre-CT sample (23%, 95%CI=[15-31%], median 2 cells, range [1-17 cells]). CTC positivity rates were 17% in 86 post-CT samples and 27% in all 118 patients. Persistence of CTC at the end of CT was not correlated with treatment response. After a short median follow up of 18 months, the presence of CTC (p=0.017), hormone receptor negativity and large tumor size were independent prognostic factors for shorter metastasis-free survival. *Conclusion:* The CellSearch® system can detect CTCs in 27% of patients receiving neoadjuvant chemotherapy, using a low cut-off of 1 cell/tube. CTCs detection was not correlated to the primary tumor response but is an independent prognostic factor for early relapse.

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DAVID PAUL HANSEMANN, CHROMOSOMES AND CANCER

Leon P. Bignold, Brian Coghlan and Hubertus Jersmann

University of Adelaide, SA 5005, Australia

Chromosomes were described, named and identified as the basis of the cellular hereditary material in the 1870s-80s. In the same period Weismann's theory that 'differentiation' (conversion of blastula cells into the various types of adult tissue cells) occurs by progressive loss of chromosomes during mitoses of the cells was popular. Also, studies of chromosomes during oogenesis showed that the 'polar bodies' associated with this process involve loss of chromosomes from the egg cell. In 1890, David Paul Hansemann (1858-1920) noted asymmetric mitoses in cancer cells while recognising loss of tissue differentiation and increased capacity for independent existence (*i.e.* "autonomy" – ability to grow in remote tissues and form metastases) as features of cancer cells. Probably because Virchow insisted that tumour formation must involve only an abnormality of a "physiological" tissue process (not a new process), Hansemann thought that oogenesis might be a counterpart of this particular combination of changes because (i) the egg loses chromosomes as it ripens (ii) it is less 'differentiated' than ovarian epithelial cells (in fact, it is 'de-differentiated') and (iii) the egg can survive for days free in the endometrial cavity. Hansemann called the normal process "anaplasia", and proposed that it could occur to variable degrees in different cases of tumour according to the degrees of chromosome imbalance in the tumour cells. He considered that populations of chromosomally unbalanced cells would arise, some of which have the (ovum-like) anaplastic features, but still have features of the cell type from which they arose.

Hansemann's ideas were criticized by many authors (summarized in (1)) but several authors (Hauser, 1903; Moore and Farmer, 1904; and Boveri, 1914, see (1)) produced theories of tumors involving alterations of cellular hereditary material. None of these was as comprehensive as Hansemann's, and Boveri's in particular was largely based on only one mitotic abnormality (quadripolarity) and one new cell

biological observation (wandering of cells of the blastomere in doubly-fertilized - 'dispermic' - eggs) (1). Also in the early twentieth century, the directions of cancer research moved towards investigating Mendelian genetics in relation to tumours, and the mechanisms of action of viral, physical and chemical carcinogens. Only in the last 30 or so years, have the roles of chromosomal abnormalities in tumour formation – which were first studied by Hansemann – again received significant attention.

1 Bignold LP, Coghlan BLD, Jersmann HPA. David Paul von Hansemann: Contributions to Oncology. Context, Comments and Translations. Birkhäuser, Basel, 2007.

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DNA-PROTEIN CLAMP DYSFUNCTION AS A MECHANISM OF CHROMOSOMAL ABBERATIONS INDUCED BY X-RAYS AND ALKYLATING AGENTS IN CANCER

Leon P. Bignold

University of Adelaide, SA 5005, Australia

Chromosomal abnormalities occur spontaneously in cancer cells. However also, they are inducible in normal cells by agents which affect covalent bonds relating only to DNA (*e.g.* bromodeoxyuridine), only to proteins (*e.g.* etoposide), to DNA and proteins (*e.g.* X-rays, alkylating agents) or to neither DNA nor proteins (*e.g.* higher phenols and acridines). Although it does not explain non-genotoxic clastogens, a widely held theory of chromosomal aberrations (1) is that the agents cause double strand breaks during interphase, and translocations etc arise if two breaks are followed by inappropriate rejoinings to concurrently doubly broken DNA strands from the same or another chromosome.

In recent years, it has become clear that (i) during interphase, DNA strands are clothed by chromatin proteins, and so are rarely so 'proximate' that two fibers could interact as a result of one ionizing event (2); (ii) DNA synthesis occurs in intranuclear 'factories', where clusters of DNA polymerase complexes act on 'proximate' and 'bare' DNA strands; (iii) several steps of DNA synthesis, proof-reading, mismatch and other repair involve active strand-breaking, including 'double-strand breaking' by nucleases especially at the 'replication fork' and during various repair mechanisms (3) and (v) all processes of synthesis or repair of DNA involve complexes of proteins which maintain strand alignment (DNA-protein 'clamps').

Here, it is suggested that DNA damage caused at any time in interphase including G2 leads to double and single strand breaking by repair or synthesis-related enzymes. While these strand breaks are in existence, 'clamp protein' function may fail, either due to excessive persisting abnormal DNA or due to damaged clamp proteins (many of which are present in the nucleus throughout the cell cycle) or both. This results in simple chromosome "breaks". However also at this point, the strands are being processed naked of other proteins, so that when the clamps on two adjacent DNA strands fail, swapping of strands may occur. If the swap involves another part of the same arm of a chromosome, an 'intrachange' occurs; if to another chromsome entirely a 'translocation' ('factories' are not chromosome-specific).

This hypothesis that the 'DNA-protein clamp complexes' are the 'target' of agents which cause aberrations would account for aberration-causing agents which do not react with DNA. Mutation(s) of clamp protein genes may contribute to chromosomal aberrations in cancer.

1 Genetics *23*: 494, 1938.
2 Mut Res *512*: 93, 2002.
3 DNA Repair (Amst.) *5*: 404, 2006.

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^{177}LU RADIOLABELLING AND QUALITY CONTROL OF PAMAM DENDRIMERS MODIFIED WITH PYRIDINE-N-OXIDE DERIVATIVE OF DOTA

V. Biricova, A. Laznickova, P. Hermann, M. Polasek and I. Lukes

Veronika Biricova, Francisciho 27, Levoca 054 01, Slovakia

Dendritic highly branched polymeric materials seem to be potential antitumour drug carriers. Their huge surface areas as well as an ability to encapsulate guest molecules in the macromolecule interior make them useful for cancer diagnosis and treatment. The new conjugates of pyridine-*N*-oxide derivative of DOTA, 10-[(4-carboxy-1-oxidopyridin-2-yl)methyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, with ethylenediamine-core polyamidoamine (PAMAM) dendrimers of the first and fourth generations (containing 8 and 64 amino groups on their surface) were prepared and radiolabelled with lutetium-177. The non-radioactive lutetium chloride was concurrently used with radioactive isotope Lu-177 to fully saturate the free ligand groups on the surface of the dendrimers. To obtain a higher saturation degree of radiometal, the reaction mixture was incubated at 40˚C overnight. Diethylenetriaminepentaacetic acid was added to the mixture before analysis to scavenge excess of lutetium possibly non-specifically bound to the dendrimers. Radiolabelled products were analysed using gel permeation chromatography (GPC) and polyacrylamide gel electrophoresis (PAGE). We have produced and separated the product in a sufficient quality. Radioactivity of the samples was measured on gamma spectrometer. PAGE analysis of products indicates that radiolabelled conjugates with the dendrimers have negative charges. This study clearly shows that gel permeation chromatography is an effective technique for separation and consecutive quality control of the PAMAM

dendrimer conjugates and polyacrylamide gel electrophoresis is a suitable method for its partial characterisation.

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MANIPULATION OF GLIOMA CELL SURFACE GANGLIOSIDES: POTENTIAL THERAPEUTIC IMPLICATIONS

Suzanne Birks[1], John Danquah[2], Richard Hitchman[2], Linda King[2], Reinhard Vlasak[3], Darek Gorecki[1] and Geoffrey J. Pilkington[1]

[1]Cellular and Molecular Neuro-oncology Research Group Institute of Biomedical and Biomolecular Sciences School of Pharmacy and Biomedical Sciences, University of Portsmouth, St Michael's Building, White Swan Road, Portsmouth, Hampshire, PO1 2DT;
[2]School of Life Sciences, Oxford Brookes University, Headington Campus, Gipsy Lane, Oxford OX3 0BP, UK;
[3]Applied Biotechnology, Department of Cell Biology, University Salzburg, A-5050 Salzburg, Austria

Background: The ganglioside GD3 is highly expressed in the embryonic brain but markedly decreases in latter stages of development. It plays a pivotal role in the regulation of cell proliferation and migration. In neoplastic cells, GD3 expression is once again up-regulated but becomes acetylated at the terminal sialic acid residue to form 9-*O*-acetyl GD3 (GD3A). Whilst the accumulation of GD3 in cell cytoplasm induces mitochondrially mediated apoptosis, this phenomenon does not occur when GD3 is acetylated. GD3A appears to promote tumour cell growth and survival. However, exogenous addition of the enzyme acetylesterase deacetylates GD3A and restores pro-apoptotic native GD3. The baculovirus *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) can efficiently transduce mammalian cell lines leading to stable and transient gene expression whilst remaining non-pathogenic and may be a suitable expression system for *in vivo* therapeutic targeting of GD3A. *Aims:* Target GD3A expressing brain-tumour cells with acetylesterase *in vitro* by exogenous addition and transfection with influenza c virus HE protein cDNA to assess the effect on levels of GD3/GD3A expression, apoptosis and invasion and to determine whether the baculovirus AcMNPV can transduce human brain-tumour cells and may therefore be a suitable vector system for therapeutic targeting of GD3A. *Materials and Methods:* Recombinant acetylesterase from influenza C virus, produced in SF9 cells and supplied by R. Vlasak, was used at 10 mU in all assays. Cells used in all assays were from a glioblastoma multiforme biopsy-derived cell culture. The pCHE4 plasmid encoding the acetylesterase (hemagglutinin esterase (HE) protein) from the influenza C/California/78 virus was also supplied by R. Vlasak. Transductions with the baculovirus were performed by J. Danquah using recombinant baculovirus AcEGFP-VP39 at multiplicity of infection (MOI) 50, cells were then fixed and lablelled with anti-VP39. GD3/GD3A expression studies were carried out by incubating cells with acetylesterase followed by staining with anti-GD3, anti-GD3A and analysis by flow cytometry. Apoptosis assays were performed by incubating cells with acetylesterase followed by either staining with Annexin V or the JC-1 probe and analysis by flow cytometry or running lysed cells on SDS-PAGE and Western immunoblotting for the release of cytochrome *c* and caspase-9 and caspase-3. Invasion assays were carried out either by the modified transwell-Boyden chamber invasion assay where cells applied to the upper surface were suspended in SFM + acetylesterase (SFM + PDGF was used as a chemoattractant) or by live cell imaging for 24 hours in the presence of acetylesterase. *Results:* The exogenous addition of acetylesterase results in a decreased expression of GD3A and an increase in the expression of GD3. This is correlated to a decrease in cell survival since the percentage of dead/apoptotic cells was significantly more when pre-treated with the enzyme. *Discussion:* Since exogenous addition of acetylesterase only targets GD3A on the surface of the brain tumour cells, we expect to see much more convincing effects when the enzyme is active within the cell. Since the baculovirus transductions are so far very promising we hope to start work cloning acetylesterase cDNA into the baculovirus.

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DETECTION AND REPAIR OF A REPLICATION FORK ENCOUNTERED SINGLE-STRAND BREAK – A MODEL SYSTEM FOR DAMAGE GENERATED *VIA* TOPOI-DNA CLEAVAGE INTERMEDIATES

Ida Nielsen, Iben Bach Bentsen, Anni H. Andersen and Lotte Bjergbaek

Department of Molecular Biology, University of Aarhus, C.F. Möllers Allé building 130, DK-8000 Aarhus, Denmark

The S-phase is a period of great vulnerability for the genome of eukaryotic cells. Many complicated processes are undertaken during this critical phase of the cell cycle, including the complete unwinding and duplication of enormously complex DNA molecules. During this process, replication forks frequently encounter obstacles that impede their progression. The replication fork must be able to cope with such obstacles as this otherwise it can cause replication fork stalling and eventually lead to collapse of the replication fork with the result of single- or double-strand break formation. Replication fork stalling or damage will therefore compromise genomic integrity if not properly processed.

Single-strand breaks (SSB) are frequent endogenous DNA lesions in cells and impose a great danger to an advancing

replication fork. SSBs can be induced directly by free radicals or damaged bases. Furthermore, poisons against DNA topoisomerase I (TopoI) will also generate nicks in the DNA as the enzyme gets trapped in a cleavage intermediate being covalently linked to the DNA at the 3' end of the nick. Not only do anticancer drugs such as camptothecin stimulate TopoI-DNA cleavage intermediates, but so do endogenous and exogenous DNA lesions including UV-induced base modification, guanine methylation and oxidation, base mismatches and abasic sites. It is believed that TopoI-DNA cleavage intermediates damage DNA and kill cells by generating replication-mediated double-strand breaks (DSB) and by stalling transcription complexes. Topoisomerase I is an abundant enzyme and is furthermore known to travel along with the DNA replication machinery, possibly removing topological strain in front of the replication fork, it is therefore likely that TopoI can induced damage arise in every S-phase.

In order to identify and characterize pathways engaged in repair of topoisomerase-induced DNA damage, we have designed an *in vivo* system where we can induce an SSB at a single locus in the genome. The induced SSB mimics a TopoI-DNA cleavage intermediate. Using this system, we have a unique opportunity to examine which stress response factors get activated and to study in detail how the DNA replication fork copes with these challenges. Data obtained with the system will be presented.

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RESISTANCE OF FRESHLY ISOLATED PERIPHERAL BLOOD LEUKOCYTES TO VIRAL INFECTIONS AS ONE OF THE INNATE IMMUNITY MECHANISMS IN HEALTH AND DISEASE–POSSIBLE THERAPEUTIC EFFECT OF PLANT PREPARATIONS

Z. Błach-Olszewska[1], E. Zaczyńska[1], M. Sochocka[1], B. Jatczak[1], A. Wiśniewska[1], I. Frydecka[3], M. Kiełbiński[2] and J. Robaczyński[3]

[1]Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław;
[2]Department of Hematology, Medical University, Wrocław;
[3]Department of Fertility and Obstetrics, Medical University, Wrocław, Poland

The aim of this study was to compare the resistance of healthy blood donors leukocytes *ex vivo* to viral infection with the resistance of leukocytes isolated from cancer patients. The resistance is individually differentiated, age-dependent and relies on TNFα and IFNs (α, β, γ).

The degree of resistance was measured by using a direct method of infection of leukocytes with vesicular stomatitis virus (VSV), which is an indicatory virus in the test. The lack of VSV replication by infected leukocytes (0-1 log $TCID_{50}$) was taken as a complete resistance; a low level of VSV (2-3 log) for partial, and a high VSV titer (4 or more log) for no or very low resistance.

Results showed statistically significant differences between the resistance of the healthy and leukemia groups at diagnosis and the importance of the degree of the resistance for induction of remission after chemotherapy and survival time. Very low immunity was also found in leukocytes of cancer patients (ovarian and endometrial cancer). With respect to potential therapeutic usefulness, intensification of the resistance by plant preparations from *Scutellaria baikalensis, Ginkgo biloba, Echinacea purpurea* and donepezil (used in Alzheimer's disease therapy) was found.

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ASSESSING THE ROLE OF HUMAN PAPILLOMAVIRUS (HPV) ONCOPROTEINS IN REGULATING CELL-MEDIATED IMMUNITY: IMPLICATIONS FOR CERVICAL CANCER

Oliver Watherston[1], Paul Bowles[1,2], Graham Bottley[1], Bodil Norrild[3] Graham P. Cook[2] and G. Eric Blair[1]

[1]Institute of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, LS2 9JT, UK,
[2]Leeds Institute of Molecular Medicine, St James's University Hospital, Leeds LS9 7TF, UK;
[3]Health Science Faculty, University of Copenhagen, DK-2200, Copenhagen, Denmark

Previous work from this laboratory has investigated the modulation of cell surface Major Histocompatability Complex class I (MHC class I) expression by the E7 oncoprotein from high risk Human Papillomavirus 16 (HPV16). We utilized two complementary cell-based systems to induce or knock-down expression of the E7 protein, and determined changes to MHC class I expression. We have already shown that siRNA-mediated inhibition of E7 in HPV16- and HPV18-transformed cells results in significant up-regulation of cell-surface MHC class I molecules. We have also shown that induction of HPV16 E7 using a tetracycline-repressor system results in a significant down-regulation of cell surface MHC class I molecules. In addition, analysis of total cell MHC class I heavy chain protein by Western blotting has confirmed the results obtained by flow cytometry on cell-surface MHC class I. Interestingly, total cellular levels of the invariant chain of the MHC class I heterodimer, β2-microglobulin, appeared to be unaffected by E7 expression, suggesting a selective effect on molecules encoded in the MHC locus (since *β_2-microglobulin* is located outside the MHC locus). We are currently examining levels of other components of the antigen processing and presentation pathway (TAP, LMP2, LMP7 *etc*).

We have also examined the functional implications of MHC class I down-regulation by HPV E7 using a co-culture system with peripheral blood Natural Killer (NK) cells. These co-culture experiments revealed that the cell surfaces changes in MHC class I levels mediated by E7 observed have a significant effect on susceptibility to NK mediated lysis, consistent with "the missing self hypothesis". Thus, E7-expressing cells having reduced MHC class I were less resistant to NK cell mediated lysis. Conversely, E7-knockout cells having increased cell surface MHC class I were more resistant to NK cell lysis. We are now investigating inhibitory and activating NK cell ligands on HPV-transformed cells to elucidate the mechanism of the NK interaction with these malignant cervical cells.

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NESTED MORPHOLOGY IN UROTHELIAL CARCINOMAS: AN ELEMENTARY LESION SHARED BY MANY DIFFERENT SUB-TYPES OF URINARY BLADDER CANCER

Enrico Bollito

Division of Pathological Anatomy, University of Turin at San Luigi Gonzaga Hospital- Orbassano, Turin, Italy

The incidence of bladder cancer has markedly increased, mainly as a result of smoking, particularly within women.

Recently, the new 2004-WHO classification of these tumors has re-opened the debate on many aspects of these lesions, improving the distinction of the different variants of bladder cancer. The existence of a group of bladder cancers that seems to have special features has become more evident: they are carcinomas frequently showing an inverted or endophytic growth pattern, and presenting heterogeneous histological aspects and different grades, sometimes high, of aggressiveness, but they seem to share the same elementary lesion: the nest.

The group of urothelial lesions with nested morpholgy may include different benign or malignant conditions ranging from von Brunn nests, inverted urothelial papillomas to inverted, nested type, microcystic and micropapillary carcinomas. Among the latter, some malignant forms were recognized as malignant tumors having a worse prognosis than conventional urothelial carcinomas. The study of bladder cancer with nested morphology is therefore of interest for a better knowledge of the development of these tumors and because the nested variants may represent, if not a true separate sub-type, at least a separate growth pattern with possible prognostic and therapeutic implications. Further investigations, particularly with molecular techniques are needed to assess the existence of a true difference beetween tumors with nested or esophytic morphology.

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P53 FAMILY TUMOR SUPPRESSOR NETWORK LINKS MULTIPLE TUMOR SUPPRESSOR PATHWAYS: P63/P73-DEPENDENT TUMOR SUPPRESSOR PATHWAYS

Lakshmanane Boominathan

46 III Cross Street, Nehru Nagar, Mudaliarpet, Puducherry, 605 004, India

It is known for years that several tumor suppressor genes exist in a cell. However, it remains elusive whether there is any coordination among the established tumor suppressor genes to determine the fate of the cell in response to aberrant proliferative signals/DNA damage. Every gene in a cell needs to be linked to another gene through gene network. Genes that perform similar functions may be functionally linked and they could be coordinately regulated. If this is true, then genes that are classified under the title of tumor suppressor genes may not be an exception to this possibility. Hence, I propose that it is very important to understand how one tumor suppressor gene is connected to another tumor suppressor gene – through tumor suppressor network – within the cell. Any biological or chemical agent that disrupts the tumor suppressor network will result in the initiation of tumorigenesis. A single nucleotide/codon alteration can change the fate of the cell. For example, missense mutation in the tumor suppressor p53 results in cancer. The p53 and INK4s(a,b,c)-ARF tumor suppressors are the most prominent among all the tumor suppressor genes known so far. It appears that in most of the cancer cell types, if not all, acquire a mutation in the pathway leading to the induction of p53- or INK4s(a,b,c)-ARF tumor suppressors. Having realized the importance of these tumor suppressor genes, it is of paramount importance to identify other tumor suppressor genes that link both p53- and INK4s(a,b,c)-ARF genes together within the cell. With the arrival of two p53 related genes, p73 and p63, the p53 tumor suppressor family network appears to have enlarged in scope and significance. I have identified several tumor suppressor genes that link both p53- and INK4s(a,b,c)-ARF-family genes together. The bioinformatics approach was used to identify the potential tumor suppressor network. The models that emerge from this study will mainly explain how p63 and p73 function as tumor suppressors. Then, the identified tumor suppressor network will be classified to illuminate the specific tumor suppressor pathways. Additionally, the concept of "tumor suppressome" will be elaborated. In aggregate, this study was aimed to identify: a) Networks among the established tumor suppressors that directly link the p53 family tumor suppressor proteins together; b) p63/p73-dependent tumor suppressor pathways; c) the unidentified tumor suppressor networks that directly link several tumor suppressors.

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A REVIEW OF PROGNOSTIC FACTORS OF EARLY CERVICAL CARCINOMA

J. Bouda[1], L. Boudova[2], Z. Rokyta[1], B. Gabriel[3], G. Gitsch[3] and E. Stickeler[3]

Departments of [1]OBGYN and [2]Department of Pathology, Medical Faculty Hospital, Plzeň, Czech Republic; [3]OBGYN, Freiburg University Medical Center, Freiburg, Germany

Establishing prognosis is an important step for therapy planning in malignant diseases. The classical morphological prognostic factors in cervical carcinoma are: stage, type, grade, tumor volume or diameter, depth of stromal invasion, lymphovascular invasion, regional lymph node status, and positivity of surgical margins. Recently, the focus of research moved to novel factors such as oncogenes, tumor suppressor genes, oncomarkers, aneuploidy and microsatellite instability, neovascularisation, proliferative activity, HPV infection, adhesion molecules.

In this review, we analyse the role of classical prognostic factors of early cervical carcinoma and the role of novel factors such as CD 44v6 adhesion molecule, focal adhesion kinase (FAK) and hTra2-beta1 splicing factor. We also discuss the impact of prognostic factors for treatment individualisation and outline the future directions in this field of research.

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CAS MEDIATES CROSSTALK OF SRC AND mTOR SIGNALING

Kristýna Bicanová, Lucie Teglová, Daniel Rösel and Jan Brábek

Department of Cell Biology, Faculty of Science, Charles University in Prague, Prague, Czech Republic

mTOR signaling was found to contribute to various aspects of Src mediated transformation, especially anchorage independent growth. CAS (Crk associated substrate) is a major tyrosine phosphorylated protein in cells transformed by v src oncogene and was found to be critical for invasion and metastasis of src transformed cells. CAS was recently documented to contribute to the activation of PI3 kinase, an upstream regulator of mTOR signaling.

To analyze the role of CAS in crosstalk of mTOR and Src pathways, we employed CAS null and CAS re expressing Src transformed MEFs. We found that CAS profoundly enhanced the phosphorylation of mTOR/Raptor substrate 4E BP1 as well as mTOR/Rictor-dependent Akt 473 phosphorylation. Using CAS null Src transformed MEFs, reexpressing CAS mutant with all 15 tyrosines in substrate domain mutated to phenylalanines, we showed that functional CAS substrate domain is necessary for 4E BP1 and to lesser extent also for Akt 473 phosphorylation.

CAS mediated invasiveness of Src transformed MEFs is accompanied by great elevation of MMP 2 activity (Brábek *et al*., 2004). We analyzed whether mTOR signaling participates in regulation of MMP 2 activity. Remarkably, we found that MMP 2 activation in Src transformed MEFs was down-regulated by mTOR inhibitors rapamycin and LY294002 and unaffected by PI3 kinase inhibitor wortmanin. In addition, we found that while mTOR inhibitors inhibited growth of Src transformed cells under both non adhesive and adhesive conditions, PI3 kinase inhibitor wortmanin inhibited the growth of Src transformed cells only under non adhesive conditions, suggesting that under adhesive conditions the crosstalk of Src and mTOR signaling is independent of PI3 kinase.

To summarize, we found that CAS mediated crosstalk of Src and mTOR signaling and that this crosstalk is at least in part PI3 kinase independent.

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MOLECULAR-TARGETED THERAPY FOR ADVANCED RENAL CELL CARCINOMA

Sergio Bracarda[1], Marta Rossi[1], Alketa Hamzay[1], Claudia Caserta[2], Valeria De Simone[1] and Lucio Crinò[1]

[1]Department of Medical Oncology, Ospedale S. Maria della Misericordia, Perugia; [2]Department of Medical Oncology, Ospedale S. Maria, Terni, Italy

Metastatic advanced renal cell carcinoma (mRCC) is historically refractory to conventional available treatments such as radiotherapy and chemotherapy. As a result, until recently, immunotherapy (interleukin-2 and or interferon-α) has been considered the treatment of choice for advanced disease.

Recent advances in understanding the biology and genetics of RCC have led to the identification of a number of molecular targets, with several novel drugs developed for these targets, particularly tumor-associated angiogenesis, a key step in the pathogenesis and progression of RCC.

Several angiogenic growth factors are highly expressed in RCC, but vascular endothelial growth factor (VEGF), because of the inactivation of the von Hippel-Lindau (VHL) tumor-suppressor gene and the subsequent activation of the hypoxia response pathway, and mammalian target of rapamycin (mTOR), for its central metabolic and angiogenic role, are of particular importance. As a consequence, VEGF-targeted therapies and mTOR inhibitors have been identified as promising treatment strategies in patients with mRCC. To date, three main treatment approaches have emerged: VEGF ligand blockade, through anti-VEGF monoclonal antibodies (such as

bevacizumab); VEGF signal blockade, by targeting VEGF receptors with small molecule tyrosine kinase inhibitors such as sunitinib and sorafenib; and, more recently, mTOR inhibition with intravenous or oral agents such as temsirolimus (CCI-779) and everolimus (RAD001).

Recent data about clinical efficacy and significant toxicities of these agents and actual innovative treatment strategies for advanced renal cell carcinoma will be presented and discussed.

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EXPRESSION OF INSULIN LIKE GROWTH FACTORS (IGFS) IGF RECEPTORS GENES, AND IGFBP-3 IN RENAL CANCER

Michał Białożyt[1], Ryszard S.Braczkowski[2], Marta Plato[3], Bogumiła Braczkowska[4], Elżbieta Mazurek[3] and Wiesław Duda[1]

[1]Prof. Michałowski Memory Hospital Strzelecka ST. 9, 40-073 Katowice;
[2]Medical University of Silesia in Katowice, Department of Public Health Bytom, Piekarska ST. 18;
[3]Medical University of Silesia in Katowice, Department of Molecular Biology, Sosnowiec Narcyzów St.1;
[4]Medical University of Silesia in Katowice, Department of Epidemiology, 41-702 Katowice, Medyków ST.10, Poland

Insulin like growth factors I and II play important roles in regulation of cell proliferation. They both have strong promitotic and antiapoptotic effects. The actions of IGFs are mediated by their activation of the specfic cell membrane receptor with tyrosine- kinase activity called IGF-I receptor (IGF-IR). There is also another type of IGF receptor – IGF II receptor (IGF-IIR). This receptor has no tyrosine-kinase activity, and does not exert mitogenic and antiapoptotic action. The activity of IGFs is regulated by their binding proteins. Because of their mitogenic and antiapoptotic actions IGFs can act on tumor growth. There are many epidemiological studies showing that incresed circulating levels of IGFs are connected with increased risk of several cancers. Not only circulating but also locally produced IGFs, can act on tumor growth in a paracrine-autocrine way. Production of IGFs, and presence of IGF-IR has been observed in several cancer cells. IGF-I and IGF-II have strong mitogenic and antiapoptopic effects on normal and transformed kidney cells. This evidence suggests that IGFs may promote the development and growth of renal cancer. Renal cancer represents 3-4% of all human malignant neoplasms. Recent studies suggest that the incidence of RCC is increasing. About 2500 new cases of renal cancer per year are registred in Poland. The most frequent among them is renal cell carcinoma and among them 70-80% are clear cell carcinoma.Radical nephrectomy is stil the main therapy of this cancer. *Materials and Methods:* Patients qualified to radical nephrectomy, aged 25-65, were included to the study. Patients under 25, and over 60 were excluded. Production of IGFs decreases with age, being highest in young people, and strongly decreases in older patients, so this exclusion was used in order to have an homogeneous. Patients qualified for nephrectomy because of cancer were included to examined group, and patients qualified for other reason were included to the control group. All patients signed informed consent as per institutional guidelines. After radical nephrectomy tumor specimens were histopatologicaly evaluated. Only materials obtained from patients with tumors qualified as ca clarocellulare were subject to further examination. Finally 49 (age 40-65) patient were included into examined group, and 15 (age 32-63) patients to control group. Material from tumors was subsequently evaluated according to Fuhrman malignancy scale. Then specimens coming from tumors, health tissue of tumor area and from kidney nephrectomized because of other than cancer reasons were processed according to gene expression. Expressions of genes for IGFs, IGF receptors and IGFBP were evaluated by QRT-PCR (Taqman). Data analysis was performed by means of standard statistical procedures using STATISTICA 7.1. *Results:* IGF-I. Only in 19 samples of tumor tissue and in 3 samples of tumor area, expression of IGF-I gene was observed. The expression in tumor is inversly correlated with tumor grade. IGF-II. Expression of IGF-II gene was observed only in tumors with high Fuhrman grade (3 and 4). Expression was observed also in 3 samples from tumor area and in 5 samples from kidney nephretomized because of non cancer reasons. IGF-IR. Expression of IGF-IR gene was noted only in 2 samples from non cancer kidney and only in 2 (of 16) samples from the tumor area of patients with

cancer Fuhrman grade 1. Expression was observed in all other cases. Expression in cancer tissue with Furhrman grade 2, 3 and 4 was higher (statistically significant) than in cancers with Fuhman 1. IGF-IIR. Expression of IGF-IIR gene was present in all samples from non cancer tissues. Expression was observed only in five samples from a healthy area from kidney with cancer (4 with Fuhrman 1 and 1 with Fuhrman 3). Expression was observed in 3 tumors with Furhman 1. In all other tumors no expression of IGF-IIR was observed. IGFBP-3. IGFBP -3 gene expression was not present in all samples from non tumor kidney, and was observed in all (excluding 2) samples from cancer and cancer area.

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MODULATION BY STIMULI TREATMENT OF CAM EXPRESSION, PROLIFERATION AND APOPTOSIS ASSOCIATED TO CERVIX TUMOR CELLS

Lorelei I. Brasoveanu[1], Gabriela Anton[2], Mirela Hirt[1] and Mihai Stoian[2]

[1]Center of Immunology, Bucharest;
[2]Institute of Virology "Stefan S. Nicolau", Bucharest, Romania

Proliferation of mammalian cells is tightly regulated by multiple environmental influences, adhesion to extracellular matrix (ECM), cell-cell adhesion and soluble factors (cytokines or activators of kinases); loss of adhesion generally results in complete G1 phase cell cycle arrest or apoptosis. In contrast, formation and spread of tumors are closely associated with decreased dependence on adhesion for growth and survival. Cervical cancer represents the second malignancy as frequency among women and is due by persistent infection with high risk human papilloma viruses (hrHPV). Co-expression of certain cell adhesion molecules (CAMs) by cervix tumor cells, which might be involved in cellular interactions, changes in adhesivity and cellular mobility, could influence the aggressiveness and metastatic potential of a certain tumor. The present study focused on the potential influence of treatment with several stimuli as cytokines or drugs(doxorubicin) on proliferation by cell cycle phases and apoptosis of cervix tumor cells using as models the HPV^+ immortalized CaSKi and HeLa cell lines. In addition, the effect of stimuli treatment on expression of several CAMs (E-cadherin, ICAM-1, MUC-1, VCAM-1) was analyzed. Expression of cellular associated antigens was evaluated by indirect immunofluorescence followed by flow cytometry analysis. Percentages of apoptotic cells were detected by using annexin V/FITC and propidium iodide (PI) double staining, while progression through cell cycle phases was evaluated by using PI staining. Data obtained showed that both the expression of CAMs associated to the human cervix cell lines taken under study and proliferation thorough cell cycle phases or apoptosis were differentially influenced depending on the stimuli used. All these data bring new information regarding CAMs and proliferation profile associated to cervical tumor cells and their possible involvement in regulating the interaction between tumor cells and host immune system, leading to new antitumor gene- and immuno-therapeutical strategies.

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ROLE OF PKC ISOFORMS IN THE FORMATION OF METASTASIS

Walburgis Brenner, Isabelle Greber, Silke Beitz, Justine Gudejko-Thiel, Frederik Roos, Joachim W. Thüroff

Department of Urology, University of Mainz, Germany

Background: The most common reason for death in cancer patients is the development of metastases. The formation of metastases depends on adhesion of tumor cells to the endothelium, migration into the subendothelial tissue and proliferation *versus* apoptosis in the secondary organ. These processes depend on many intracellular signaling mechanisms, which are partly regulated by protein kinase C (PKC). Different PKC isoforms have various regulatory effects in processes of metastases. We analysed the role of PKC isoforms in progression and metastases of renal cancer. *Materials and Methods:* The expression of all PKC isoforms was determined in the human renal carcinoma cell line CCF-RC1 and in tissue specimens of renal tumor and normal renal tissue. For functional analyses, cells were treated with the PKC specific inhibitors RO31-8220, GF109203X, GÖ6976 and Rottlerin. Afterwards, cell adhesion on monolayer of human umbilical vein endothelial cells (HUVECs) was investigated. The migration was quantified using a microchemotaxis chamber and proliferation was determined by BrdU incorporation. The cellular expression and phosphorylation of beta-1 integrins and the downstream target focal adhesion kinase (FAK) was quantified by Western blot. The expression of beta-1 integrins on the cell membrane was quantified by flow cytometry. *Results:* With the exception of PKC lambda and theta, all PKC isoforms were expressed in the renal carcinoma cell line CCF-RC1, in renal tumor specimens and in normal renal tissue. Cell adhesion and proliferation were unaffected by all PKC inhibitors. Cell migration was reduced after treatment with GF109203X or Rottlerin down to 69% or 29% of untreated cells, respectively. The cellular expression and phosphorylation of beta-1 integrins and FAK are also inhibited by Rottlerin, a PKC delta specific inhibitor. In contrast, the expression of beta-1 integrins on the cell membrane was most potently reduced by RO31-8220 down to 8% of untreated cells. *Conclusion:* Treatment with PKC inhibitor GF109203X demonstrated a clear influence on cell migration. PKC delta is the only isoform which is inhibited by GF109203X but not by RO31-8220 or

GÖ6976. Since also Rottlerin, a strong PKC delta inhibitor, reduces cell migration, it is feasible to assume that PKC delta regulates migration, but not adhesion and proliferation of renal carcinoma cells. On the other hand, the only PKC isoform which is inhibited by RO31-8220, but not by one of the other used PKC inhibitors, is PKC epsilon, this isoform seems to regulate the membrane expression of beta-1 integrins, although it has no influence on cell migration. Our results suggest that the inhibition of cell migration depends on the regulation of the activity of beta-1 integrins and its downstream target FAK.

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THE USE OF MULTIPLEXED APTAMER MEASUREMENTS OF PROTEINS FOR THE DIAGNOSIS AND SCREENING OF LUNG CANCER

Edward N. Brody

SomaLogic, Inc., 2945 Wilderness Place, Boulder, Colorado, 80301, USA

Aptamers are single stranded nucleic acids that are evolved through the SELEX procedure to bind tightly and specifically to cognate proteins. Recent advances in the SELEX protocol have led to a new kind of aptamer (called SLaptamers); SLaptamers have very high affinity and specificity for cognate proteins, and not others, due to very long dissociation rates (>30 min) for the cognate protein. By coupling the new protocol with the use of chemically modified bases in starting libraries, SomaLogic has evolved new specific binding reagents for multiplexed measurements of proteins in blood and other biological fluids. Because one SLaptamer can give the same specificity and sensitivity that requires two antibodies in the ELISA format, SLaptamer arrays reduce cross-reactivity between reagents in a multiplexed array; there is essentially no limit on how many measurements can be made simultaneously. SomaLogic now has the capacity to measure over 600 proteins in blood. Using blood samples from smokers without cancer, early-stage (I and II) lung cancer, and late stage (III) lung cancer, measurement of serum proteins has detected biomarkers which, within this study, can discriminate smokers without cancer from both early and later stage lung cancer.

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FUNCTIONAL FOOD SCIENCE AND SELENIUM IN CANCER PREVENTION. A SHORT-TERM INTERVENTION TRIAL WITH SELENIUM-ENRICHED FOOD: EFFECTS ON BIOAVAILABILITY AND OXIDATIVE DEFENCE REGULATION

M. Rondanelli[1], A. Opizzi[1], A. Giacosa[2] and G. Broich[3]

[1]Dipartimento di Scienze Sanitarie Applicate e Psicocomportamentali, Sezione di Nutrizione, Azienda di Servizi alla Persona di Pavia, Università di Pavia; [2]Dipartimento di Gastroenterologia, Policlinico di Monza; [3]IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

Results from several but mostly small observational studies as well as the secondary analysis of an intervention trial provide support for a chemopreventive effect of selenium against cancer, in particular prostate, lung, liver and colorectal cancers. Several cancer preventive mechanisms have been described and it is likely that selenium acts through multiple pathways. In particular, the anti-oxidative and anti-inflammatory effects mediated through activity of seleno-enzymes are discussed, given the relevance of oxidative stress and inflammation in these cancers. The effect seems to be strongest in those individuals with the lowest Se status. There is some evidence that Se may affect not only cancer risk but also progression and metastasis. Current primary and secondary prevention trials of Se are underway in the USA, including the Selenium and Vitamin E Cancer Prevention Trial (SELECT) relating to prostate cancer, although a large European trial is still desirable given the likelihood of a stronger effect in populations of lower Se status. If Se may protect against cancer, an adequate intake of Se is desirable. However, the level of intake in Europe and some parts of the world is not adequate for full expression of protective selenoproteins. Selenium levels in soil generally reflect its presence in food and the Se levels in human populations. Thus, food content is influenced by geographical location, seasonal changes, protein content and food processing. Periodic monitoring of Se levels in soil and food is necessary. Diet is the major Se source and approximately 80% of dietary Se is absorbed depending on the type of food consumed. Se bioavailability varies according to the Se source and nutritional status of the subject, being significantly higher for organic forms of Se. Se supplements can be beneficial for subjects living in regions with very low environmental levels of Se. Several strategies have been followed: (1) employment of Se-enriched fertilizers; (2) supplementation of farm animals with Se; (3) consumption of multimicronutrient supplements with Se. Nevertheless, detailed investigations of possible interactions between Se supplements and other food components and their influence on Se bioavailability are needed. Given this background, in a 3 week randomised, double-blind study, 7 healthy young women (age 25±2 years, Body Mass Index 23±1) supplemented their usual diet with Se-enriched food and 7 were supplied with a similar diet but not supplemented with Se. Before and after 21 days of supplementation serum Se level and erythrocyte glutathione peroxidase (GPX) activity were evaluated. Serum Se levels were significantly increased in the intervention group (99.3±17.7 microgram/ml at time 0 and 114.1±28.6 microgram/ml at time +21 day, $p<0.003$ by Man-Withney test), while the control group did not show any significant difference (98.2±18.7 microgram/ml at time 0 and 100.2±23.4

microgram/ml at time +21 day). Similar results were observed when erythrocyte glutathione peroxidase (GPX) activity was evaluated. The group supplemented with food enriched Selenium showed a significant increase in GPX activity after 21 days of intervention (19.1±3.1 at time 0 and 22.1±4.0 at time +21 day, $p<0.01$), while its value did not change in the control group (20.2±2.8 at time 0 and 19.1±2.9 at time +21 day). Moreover, the follow-up of the supplemented subjects, showed that ten days after the end of the supplementation, both the serum selenium levels and GPX activity decreased and did not differ significantly when compared with values at time 0. In conclusion, this study demonstrated the bioavailability of Selenium supplemented through fortified food, thus proving that this approach could be helpful to favour the optimal intake of Selenium in the population and therefore to favour cancer prevention.

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THE SERINE/THREONINE KINASE AKT ACTIVATED BY FIBROBLAST GROWTH FACTOR RECEPTORS FROM OESTROGEN-INDEPENDENT BREAST CANCER CELLS REGULATES THE G_2/M CHEKPOINT

Edith Browaeys-Poly[1], Arlette Lescuyer[1], Dominique Perdereau[2], Jean Pierre Vilain[1], Anne-Françoise Burnol[2] and Katia Cailliau-Maggio[1]

[1]Université des Sciences et Technologies de Lille, Laboratoire de Régulation des Signaux de Division, EA 4020, IFR 147, SN3, Villeneuve d'Ascq Cedex, France; [2]Institut Cochin INSERM U567-CNRS UMR 8104-Université René Descartes, Département Endocrinologie, Métabolisme, Cancer 24 rue du Faubourg Saint-Jacques, 75674 Paris, France

Hormone-independent breast cancers proliferate in response to "fibroblast growth factor". The oestrogen-independent breast cancer cell line, MDA-MB-231, expresses fibroblast growth factor receptors (FGFRs), and secretes FGF1 that exerts an autocrine mitogenic effect. Analysis of FGFRs signalling is rendered difficult by the concomitant expression of several tyrosine kinase receptors and their shared transduction cascades. To specifically investigate FGFR signalling, we used a biological system the *Xenopus* oocyte, a giant cell, devoid of endogenous FGFRs, that allows microinjections and expression of various RNAs. This paradigm offers a powerful experimental approaches to question cascade transduction regulation in relation to the cell cycle G_2/M checkpoint. Oocytes expressing FGFRs from MDA-MB-231 enter in M phase, after stimulation by exogenous FGF1. This G_2/M transition involves Ras-dependent and Ras-independent cascades. The phospholipase C gamma (PLCγ) and the serine/threonine kinase (Akt), two enzymatic effectors activated by FGFRs, are involved in breast cancer growth. In oocytes expressing FGFRs from MDA-MB-231, inhibitors of PI3Kinase-Akt pathway (Wortmannin, LY 294002, N terminal SH2 domain of p85 PI3Kinase) and an inhibitor of PLCγ (a mimetic peptide of the SH2 domain) block FGF1-FGFR transduction. Activation of PLCγ is the result of a direct binding to the FGFRs p(Y)766 site. A PLCγ/calcium dependent chlorure current, measured by electrophysiological techniques, starts 2 to 4 minutes after FGF1 addition, and displays a duration of 20 minutes. A kinetic analysis shows that PLCγ is phosphorylated on tyrosine 5 minutes and on serine 30 minutes after FGFRs activation. PLCγ immunoprecipitations show that the serine phosphorylated PLCγ is associated with active phosphorylated Akt (serine 473). A PLCγ mimetic peptide of the SH3 domain disrupts the PLCγ-Akt interaction, the serine phosphorylation of PLCγ and favours Akt binding to an other downstream target: Chfr, a mitotic checkpoint protein deregulated in breast cancers. Moreover, an acceleration of the G_2/M transition occurs when the Akt-PLCγ interaction is substituted for the Akt-Chfr interaction. In conclusion, in the oestrogen-independent breast cancer line, MDA-MB-231, cell cycle progression in the M phase induced by FGF receptors is controlled by a time regulated interaction of activated Akt with two partners PLCγ and Chfr.

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INCREASED CYTOPLASMIC EXPRESSION OF THE RECEPTOR (R) FOR INTERLEUKIN (IL)-23 IS A MARKER FOR EPITHELIAL PROLIFERATION, PROSTATIC INTRAEPITHELIAL NEOPLASIA (PIN), AND PROSTATE CANCER (CAP)

Dieter Mitteregger[1], Wolfgang Brozek[1,2], Martin Susani[3], Igor Stancik[1,4], Michael Marberger[1,5] and Gero Kramer[1,5]

[1]The Ludwig Boltzmann Society, Cluster Urology, Vienna; [2]Department of Pathophysiology and [3]Clinical Institute for Pathology, [5]Medical University of Vienna; [4]Department of Urology, Hospital Hietzing, Vienna, Austria

Benign prostatic hyperplasia (BPH) and prostate cancer (CaP) were found to be related to chronic inflammation and cytokine production. We previously reported the expression of the inflammatory master cytokine IL-23 and its receptor in the prostate. In this study we investigated the association between IL-23R expression and prostatic carcinogenesis.

IL-23R was localized immunohistochemically in 28 prostate carcinoma sections and 14 carcinoma-surrounding prostate sections from 20 patients, and 1 benign prostate biopsy core. Tissue IL-23R expression was evaluated particularly with regard to the sequential stages of prostate carcinogenesis, ranging from normal glands to carcinoma *in situ* and invasive cancer. IL-23R expression of 3 CaP cell lines

(PC3, LNCaP, and DU145) was compared to the benign prostate epithelial cell line PNT2 by flow cytometry, real-time PCR, immunoflourescence, and immunocytochemistry. The association between IL-23R and proliferating cell nuclear antigen (PCNA) expression was analyzed by flow cytometry, and growth effects of recombinant human (rh) IL-23 or a neutralizing anti-IL-23 antibody were assessed in a [3H]thymidine incorporation assay in PNT2 cells and all malignant cell lines.

Two expression patterns of IL-23R were identified: Cells of normal glands expressed IL-23R restricted to the luminal cell membrane and in apocrine secretion structures. Glandular sectors showing morphological signs of proliferation, atrophic glands, PIN, and prostate cancer cells displayed a clearly increased staining intensity as well as distribution of IL-23R expression over the whole cytoplasm. In accordance, 3 CaP cell lines displayed higher cytoplasmic IL-23R expression than the benign prostate epithelial cell line. 18/28 carcinomatous and 7/14 surrounding sections contained 30 (high grade) PIN lesions. One was found in the benign prostate biopsy. 100% of these were clearly marked by increased cytoplasmic IL-23R staining, as were 26/28 (93%) of prostate carcinomas. 7/26 prostate carcinoma sections showed areas of partial loss of IL-23R expression. In tissue sections, malignant and proliferating cells were characterized by a shift of IL-23R localization from the luminal membrane to the cytoplasm. Proliferation of 2/3 carcinoma cell lines and of PNT2 cells was significantly inhibited by rhIL-23, and on the contrary increased by IL-23 immuno-depletion.

IL-23R expression level and pattern is a marker for epithelial proliferation, prostatic carcinoma *in situ* and invasive cancer. IL-23R signaling from the cellular surface might relay growth inhibitory stimuli. In accordance, intracellular translocation and hence accumulation of the receptor withdraws proliferating and malignant cells from anti-proliferative signals. Interfering with the (dysregulated) IL-23R signaling pathway might therefore serve as a potential molecular therapeutic target in prostate cancer.

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EXPRESSION OF THE VITAMIN D SYSTEM AND COX-2 IN HUMAN COLORECTAL CANCER TISSUE

Wolfgang Brozek[1], Teresa Manhardt[1], Stephan Kriwanek[2], Elisabeth Bonner[3], Enikö Kállay[1] and Heide S. Cross[1]

[1]Department of Pathophysiology, Medical University of Vienna;
Departments of [2]Surgery and [3]Pathology, Hospital Rudolfstiftung, Vienna, Austria

We suggested that the balance between colonic synthesis and degradation of 1,25-dihydroxyvitamin D3 (1,25-D3) may determine concentrations of the active metabolite locally, and thus may be crucial for growth regulation of colorectal tissue and prevention of tumor progression. Chronic inflammation as indicated by over-expression of cyclooxygenase (COX)-2 often precedes and accompanies colorectal tumorigenesis. In the present study we investigated differences in mRNA expression related to the vitamin D system (CYP27B1, the synthesizing hydroxylase; VDR, the vitamin D receptor; and two splice variants of CYP24A1, the catabolic hydroxylase), and to inflammation (COX-2). These were evaluated statistically in relation to patient age, sex, tumor subsite and grading.

113 patients with colorectal tumors and 10 diverticulitis patients (controls) were randomly chosen for this study. Colorectal tissues were analyzed by semiquantitative RT-PCR and results were quantified by densitometry. Parametrical and non-parametrical methods were used for statistical evaluation. In a multivariate factor analytical model, the number of factors was chosen based on a scree plot of eigenvalues from the correlation matrix. Maximum likelihood and principal factor methods were used for generation of the initial factor matrix, which was rotated applying the varimax method. Expression of the two splice variants of CYP24A1 was significantly elevated in tumor tissues of all grades compared with that in normal mucosa ($p<0.001$), though in moderately differentiated low-grade tumors (G1/2) from the left colon (LC) of female patients reduced levels of both splice variants occurred ($p<0.05$). While expression of CYP27B1 mRNA was also increased in tumors of all grades over that of normal mucosa, statistical significance was only reached in differentiated G1/2 tumors primarily derived from male patients ($p<0.05$). However, in female patients even high-grade (G3/4) tumors exhibited increased CYP27B1 expression compared with G1/2 tumors as long as they were derived from LC ($p<0.05$), while VDR expression was lowest there ($p<0.05$). Rectal tumor tissue, especially from females ($p<0.01$), exhibited lowest CYP27B1 gene expression ($p<0.05$) and a significant decrease of VDR expression during progression towards higher grades ($p<0.05$). Significant age-related expression changes were observed in male right colon (RC) tumors: Those graded G1/2 displayed higher VDR levels with advanced age of patients ($p<0.05$), whereas in those graded G3/4 this correlation was inverse ($p<0.05$). Compared with females, tumor resections from male patients expressed COX-2 mRNA at significantly higher levels ($p<0.05$) especially in G1/2 tumors from LC ($p<0.01$), even higher than in G3/4 tumors ($p<0.05$). Subsite specificity of COX-2 expression was preserved in female patients ($p<0.01$).

Factor analysis of this evaluation indicated four mutually not exclusive patient or tumor types: i) old patients are female, ii) correlated VDR, CYP27B1, COX-2, and, to a lesser extent, wildtype CYP24 activity in tumor tissue, iii) an increase of CYP24 splice variants in undifferentiated tumors, and iv) a site-dependent increase in COX-2 expression in male patients.

Collectively, our data confirm the concept of distinct expression patterns of the vitamin D system and of a marker for inflammation in colorectal tumors, which are not only variable during progression, but are also depending on factors like tumor subsite, gender and age.

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MUTUAL ASSOCIATIONS BETWEEN AGE, GENDER, ANATOMICAL LOCATION AND MALIGNANCY OF COLORECTAL CANCERS – RELATIONSHIP TO 1,25-DIHYDROXYVITAMIN D_3 TISSUE CONCENTRATIONS

Wolfgang Brozek[1], Thomas Nittke[1], Stephan Kriwanek[2], Elisabeth Bonner[3], Meinrad Peterlik[1] and Heide S. Cross[1]

[1]Department of Pathophysiology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna; Departments of [2]Surgery and [3]Pathology, Hospital Rudolfstiftung, Juchgasse 25, A-1030 Vienna, Austria

We addressed the question whether age- and gender-related incidence of colorectal cancer (CRC) at specific anatomical subsites is associated with different degrees of malignancy by analyzing data from 107 patients presenting with colorectal tumors at a Vienna hospital in 2001/2002. Data were pooled according to anatomical subsites, *i.e.* proximal or right colon (cecum, ascending and transverse), distal or left colon (descending and sigmoid), and rectum. Parametrical and non-parametrical statistical methods were used for multivariate analysis of associations between age, sex, tumor site and tumor grading.

On the average, female CRC patients were significantly older than male patients (median age 73 *vs.* 63 yrs, $p<0.05$). The more advanced age of female cancer patients compared with males was primarily the result of age differences in proximal colon tumor patients ($p<0.05$): Men presented at a median age of 60, whereas the women's median age was 75 years. Male patients with high grade (G3+G4) cancers were significantly younger than those with low grade (G1+G2) lesions (71 *vs.* 59 years, $p<0.01$). This was not observed in women, whose median age, regardless whether they had low or high grade cancers, was around 75 years. However, at age 70-80 years more than 80% of patients with a G3 and G4 cancer were female, and at age of ≥80 years, all high grade cancer patients were women. By contrast, at age 60 years and younger, the majority, *i.e.* 67%, of G3 and G4 patients were men.

No association was found between age and a specific anatomical sub-site in male high grade cancer patients, whereas female patients with proximal colon tumors were significantly older than women with rectal cancers ($p<0.05$). Both men and women presented with high grade rectal cancers at the early mean age of slightly above 60 years.

The observation that women are protected from highly malignant cancers in the proximal colon for a long period of time, might be due to enhanced anti-proliferative potential of 1,25-dihydroxyvitamin D3 (1,25-OH_2D_3). We found (Nittke *et al.*, manuscript in preparation) that under cancer-promoting conditions, concentrations of 1,25-OH_2D_3 in human and mouse colonic tissue were in general significantly higher in females than in males, with values in the right colon exceeding those at any other site of the colorectum.

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EARLY MODIFICATION OF *C-MYC, HA-RAS* AND *P53* EXPRESSIONS BY *N*-METHYL-*N*-NITROSOUREA

F. Budán[1], Gy. Gőbel[2], I. Szanyi[2], M. Bauer[2], G. Nowrasteh[1], Zs. Varga[3], J. Cseh[4], G. Horváth[1], I. Prantner[1], P. Perjési[5], I. Ember[1] and Z. Gyöngyi[1]

[1]Institute of Public Health, University Medical School of Pécs, Szigeti str. 12, H-7643 Pécs;
[2]Institute of Otolaryngology Head and Neck Surgery, Faculty of Medicine, University of Pécs, Munkácsy str. 2, H-7621 Pécs;
[3]Country Hospital of Baranya, Department of Oncology, H-7623 Pécs;
[4]Szent György Country Hospital, Department of Oncology, H-8000, Székesfehérvár;
[5]Institute of Pharmaceutical Chemistry, University of Pécs School of Medicine, Rókus str. 2, H-7623 Pécs, Hungary

Methylnitrosourea (MNU) is a well known pluripotent direct-acting carcinogen. Formation of MNU following incubation of various meats with additional nitrite under *in vitro* acidic conditions is possible. It is possible that many species, including humans, are exposed to carcinogenic MNU, generated in their gastrointestinal tract. Previously, an animal model was developed by our research group to investigate the expression of three key onco/suppressor genes *c-myc, Ha-ras* and *p53* as early molecular epidemiological biomarkers of carcinogenic exposure or carcinogenesis induced by DMBA (dimethylbenz[α]anthracene). The aim of this study was to investigate the early effect of MNU on the gene expression levels, in order to compare the initializing role of these genes in MNU/DMBA-induced carcinogenesis. MNU is a direct-acting carcinogen which spontaneously and rapidly degrades, so any effect on the gene expression is observed within 24 hours. Our results show the maximum effect *in vivo* on the gene expression at 12 hours after the MNU treatment; on the other hand, 24 hours after the treatment, the elevated gene expressions decreased in target organs (bone marrow, lung, lymph nodes). Our results correspond to "long-term" experiments of the carcinogenic effect of MNU in different target organs. Our findings suggest that MNU has an impact

on the expression of *c-myc, Ha-ras* and *p53* genes in 12 hours, especially in bone marrow. This corresponds to the fact the DMBA has a delayed effect on the gene expression profile in comparison to MNU because DMBA is a metabolically activated carcinogen. Overexpression of these genes occurs as an early biological effect to exposure to chemical carcinogens. According to our results, these genes could indicate MNU exposure and they could be the member of genes which take part in MNU-induced tumorigenesis.

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TRANSCRIPTIONAL REGULATOR, BRN-3B/POU4F2 AND ITS TARGET GENES IN CONTROLLING GROWTH AND BEHAVIOUR OF BREAST CANCER CELLS

Vishwanie Budhram-Mahadeo

Medical Molecular Biology Unit, UCL Institute of Child Health, 30 Guilford St, London WC1N 1EH, UK

Regulation of gene expression controls all aspects of tumour growth, progression and response to treatment. By identifying the mechanisms that control such behaviour, it may be possible to specifically target these tumours to reverse growth effects. The Brn-3b transcription factor is an important regulator of growth in breast cancers and neuroblastomas because it controls expression of key target genes *e.g.* by transactivation of cyclinD1/CDK4, Brn-3b increases proliferation and anchorage independent growth *in vitro*, and tumour growth *in vivo*. Reducing Brn-3b reverses these growth effects. Brn-3b also confers drug resistance and may increase migratory potential in these cells by its effects on other target genes *e.g.* HSP 27, and gamma catenin. Brn-3b is highly expressed in >60% breast cancers and ~75% of neuroblastomas so reducing Brn-3b may be a useful for reversing its effects in such cancers. We have identified mechanisms that control the expression of Brn-3b in these cancer cells and that may be used to control its expression, under different conditions.

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PACLITAXEL/CARBOPLATINUM + CETUXIMAB AS SECOND LINE CHEMOTHERAPY FOR PATIENTS WITH RECURRENT OR METASTATIC HEAD AND NECK CANCER

J. Büntzel[1], R. Stritzel[4], A. deVries[2], R. Mücke[5], A. Garayev[1], F. Bruns[5] and O. Micke[3]

[1]Department of Otolaryngology, Municipal Hospital Nordhausen;
[2]Department of Radiotherapy, Landeskrankenhaus Feldkirch;
[3]Department of Radiotherapy, Franziskus Hospital Bielefeld;
[4]Pharmacy, Municipal Hospital Nordhausen;
[5]AKTE, Germany

Background: In patients with platinum-resistant recurrent head and neck cancer the anti-EGF-receptor antibody cetuximab could be used as a treatment option. There are only limited data about results of this therapeutic option. The objective of this study was to evaluate therapeutic benefit of this indication. *Methods:* Thirty-three patients with histological confirmed recurrent head and neck cancer (30 male, 3 female, mean age was 59±12 years) were included in this exploratory study. All recurrences had occurred after chemotherapy with platinum derivates. Thirty patients received radiation therapy during primary treatment. No surgical or radiotherapeutic option in recurrent disease was possible. Fifteen patients were suffering from local or locoregional recurrences; 18 patients had distant metastasis (17 pulmonary and 1 cerebral). The 2nd-line therapy consisted of carboplatinum (200 mg/m^2) + paclitaxel (200 mg/m^2) every three weeks (week 1, 4, and 7) and additionally cetuximab, which was given with 400 mg/. *Results*: A significant tumor response was observed in 19/33 patients (56%): 13 partial, 5 minor and one complete remission were registered. The median survival time was 7 months (range 1-14), 10 patients are still alive. Median time to progression was 5 months (range 2-8). Side-effects were rash (21/33), fever (12/33) and typical chemotherapy-induced toxicities such as neuropathy (10/33) and (pan)cytopenia (7/33). All side-effects were moderate and easily managed. *Conclusion:* The described combined chemoimmuntherapy with cetuximab and paclitaxel + carboplatinum seems to offer new strategies in 2nd- and 3rd-line chemotherapy for patients with platinum-resistant head and neck cancer, potentially overcoming primary platinum resistance.

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MYC AND GASTRIC ADENOCARCINOMA CARCINOGENESIS: STUDY IN INDIVIDUALS FROM NORTHERN BRAZIL

Rommel Rodríguez Burbano

Laboratório de Citogenética Humana, Departamento de Biologia, Instituto de Ciências Biológicas, Campus Universitário do Guamá/Universidade Federal do Pará, Av. Augusto Correa 01, CEP 66075-900, Belém, PA, Brazil

MYC is an oncogene that participates in cell cycle regulation, cell growth arrest, cell adhesion, metabolism, ribosome biogenesis, protein synthesis, and mitochondrial function. *MYC* has been highlighted as a key element of several carcinogenesis processes in humans. Many studies reported an association between *MYC* deregulation and gastric cancer. *MYC* deregulation is also observed in gastric preneoplasic lesion. Thus, *MYC* may be involved in the beginning of gastric carcinogenesis. Our results suggest that amplification is the main mechanism of *MYC* deregulation in gastric cancer. In this review, we focus on oncogene *MYC*

deregulation in gastric adenocarcinoma carcinogenesis, including its relation with *Helicobacter pylori* and clinical applications.

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CHANGES IN POST-TRANSLATIONAL MODIFICATIONS (O-LINKED GLYCOSYLATION) OF BREAST CANCER CAN AFFECT TUMOUR BEHAVIOUR AND IMMUNE RECOGNITION

Sylvain Julien, Gianfranco Picco, Julia Colman, Richard Beatson, Joyce Taylor-Papadimitriou and Joy Burchell

Breast Cancer Biology Laboratory, Research Oncology, King's College London, 3rd Floor, Bermondsey Wing, Guy's Hospital, Great maze Pond, London SE1 9RT, UK

Glycosylation is one of the most frequent forms of post translation modifications and is essential for many protein and cellular functions. Changes in the composition of glycans added to glycoproteins and glycolipids are common events in malignancy and these changes can influence the course of the disease. Dramatic alterations occur in glycans attached to serine and threonines in mucin-type O-linkage and this has been particularly studied in breast cancer. We have shown that this can be attributed, at least in part, to changes in the expression of key glycosyltransferases involved in the synthesis of O-glycans.

Greater than 90% of breast carcinomas show changes in the expression of their O-linked glycan and this raises the hypothesis that altered O-linked glycans is advantageous for breast cancer. That this is indeed the case is demonstrated by our data that show that transplantable and spontaneous murine mammary tumours expressing tumour-associated O-linked glycans grown significantly faster than the parental control. Interestingly, we have also shown that when monocyte derived dendritic cells are induced to mature and migrate to the lymph nodes, the composition of their O-linked glycans changes in a way that mirrors the change in breast cancer.

Changes in glycans attached to the MUC1 glycoprotein, a membrane mucin that carries a large amount of O-linked sugars, alters how this glycoprotein interacts with lectin-like receptors of the immune system. Indeed, certain tumour-associated glycoforms of MUC1 can bind to the macrophage galactose receptor and be internalised into antigen presenting cells, while a different tumour-associated glycoform can be immunosuppressive.

Thus changes in O-linked glycosylation can affect the behaviour of tumour cells and how tumours are recognised by the immune system. Understanding the mechanisms that induce these changes may eventually lead to identifying targets for therapeutic interventions.

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ROLE OF HEDGEHOG SIGNALING IN PROSTATE CANCER

Aubie Shaw[1,2], Jerry Gipp[1,3] and Wade Bushman[1,2,3]

[1]Department of Urology, [2]The McArdle Laboratory for Cancer Research, [3]Paul P. Carbone Comprehensive Cancer Center, University of Wisconsin, Madison, WI, USA

The Hedgehog (Hh) pathway contributes to prostate cancer growth and progression. Hh responses in tumors may arise from autocrine signaling in cancer cells or paracrine interactions with stromal cells. The evidence for autocrine and paracrine signaling will be reviewed and we will then show using a xenograft model that paracrine is sufficient to drive tumor growth. Paracrine Hh signaling occurs in embryonic prostate development and we used the developing prostate to identify mesenchymal factors that are regulated by Hh signaling. Using a xenograft model, we identified nine such factors that are aberrantly expressed in the stroma of Hh over-expressing tumors. Five of these factors correlate with Hedgehog signaling in human prostate cancer but not in benign tissue. In tumors with reactive stroma, each of the nine factors correlate with Hh signaling. We conclude that changes in the prostate stroma associated with the presence of cancer result in an altered transcriptional response to Hh ligands that mimics the growth promoting actions of the fetal mesenchyme.

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DIAGNOSTIC AND PROGNOSTIC VALUE OF CD10 IN NEUROFIBROMAS, TYPE 1 NEUROFIBROMATOSIS AND MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

Daniela Cabibi

Department of Human Pathology, University of Palermo, Italy

Neurofibromas are the most common benign peripheral tumors. They arise from the cutaneous and more rarely from the visceral, peripheral nerve sheath. In 90% of cases they are solitary, sporadic, localized, superficial tumors which are usually benign, with a very low probability of becoming malignant. In other cases they may be diffuse or plexiform and are frequently associated with Type 1 neurofibromatosis (NF1), with a higher risk of malignant transformation.

NF1, also known as Von Recklinghausen's disease or peripheral neurofibromatosis, is the most common form of neurofibromatosis, with an incidence of 1/2,500-3,000 newborns (about 1.5 million people affected throughout the world) and is one of the most frequent, progressive genetic diseases, with serious medical and social consequences. It is

transmitted in an autosomic dominant manner in about 50% of cases and is sporadic in the remaining cases due to new mutations (small deletions or puntiform mutations) on the germinal cells of paternal origin, involving the *NF1* gene, mapping on the long arm of the chromosome 17 (17q11.2). The *NF1* gene is an oncosuppressor gene with a probability of mutations 10-100 times greater than the mean of the other genes; it encodes neurofibromin, a protein that inhibits cell proliferation by modulating mitogenic pathway signaling through inactivation of p21-ras. Inactivating mutations of the *NF1* gene, with the production of truncated, inactive protein, determines hyper-activation of p21 Ras and proliferation of neoplastic cells.

The clinical presentation of NF1 is not always clear since the onset of the clinical signs is age-dependent and there is a strong variability both among patients and in the same patient during different stages of the disease, with no relation between genotype and phenotype. Due to the strong inter- and intra-familiar phenotypic variability and its different clinical courses, NF1 may sometimes require a long follow-up, lasting for more than 4 years, before being diagnosed with certainty.

The most serious risk of NF1 is the malignant transformation of neurofibromas (mainly diffuse and plexiform neurofibromas) into malignant peripheral nerve sheath tumors (MPNSTs). These represent about 5-10% of soft tissue sarcomas and are extremely aggressive, with only 34% of patients reaching a 5-year survival rate. Histologically, neurofibromas consist of Schwann cells, positive for S100 immunostain, together with perineural cells and fibroblasts, which are CD34 positive, but S100 negative.

The S100 protein is the most useful marker in the diagnosis of neurofibromas, but in some low-grade MPNSTs and in more than 2/3 of high-grade MPNSTs, it is reduced or absent. Nestin and PGP9.5 are more sensitive markers than S100, although they are not specific since they are expressed in other mesenchymal neoplasias (1, 2). As it is extremely important to reach a correct, early diagnosis, several markers have been investigated with the aim of distinguishing benign neurofibromas from those with a greater malignant potential and from MPNSTs. For example, malignant transformation of neurofibromas has been associated with CDKN2A/p16 inactivation and loss of p16 expression. *P16* is an oncosuppressor gene involved in cell cycle regulation by inhibiting CDK4, the cyclin D1-dependent kinase which enhances the progression of the cell cycle by the phosphorylation of the Retinoblastoma protein. (3, 4). Furthermore, overexpression and mutations of *P53* have been reported in high grade MPNST, but late appearance of them precludes their use as predictive markers of malignancy (3, 5).

Recently, the CD10 antigen, (also known as neutral endopeptidase (NEP), neprilisine or CALLA Antigen), first identified on the precursors of B and T lymphocytes, has been found in several hematopoietic neoplasias (B and T acute lymphoblastic leukaemias, follicular lymphoma and Burkitt's lymphoma) and in some non-hematopoietic neoplasias, such as renal cell carcinoma, endometrial stromal sarcoma, melanoma, prostatic cancer and mesenchymal neoplasia (6-10) In many tumors, such as melanomas, gastric cancer and breast cancer, a relation between CD10 expression and a greater potential of neoplastic invasiveness has been observed, probably related to the similarity of CD10 to the matrix metalloproteinases (MMPs) in creating a micro-environment facilitating neoplastic invasion (8, 11, 12). CD10 is normally expressed in breast myoepithelial cells, in kidney proximal tubules and glomerular cells, in the apical membranes of intestinal gland epithelium, in stromal cells of the endometrium and bone marrow, in biliary canalicules of the liver, in mesenchymal dermal cells (fibroblasts and dendrocytes) and, noteworthy, in the normal myelin sheath of peripheral nerves (13). Nevertheless, CD10 expression in lesions arising from the peripheral nerve sheath has still not been sufficiently investigated.

We retrospectively performed an immunohistochemical assay, by using CD10, S100 and CD34 antibodies, on formalin-fixed, paraffin-embedded seriated sections from 38 neoplastic lesions, 18 of which (Group A) consisted of localized, sporadic, solitary neurofibromas, and 20 (Group B) consisting of MPNSTs and of neurofibromas with a higher risk of malignant transformation (myxoid, plexiform, diffuse with atypias). In group B, 7 patients had a well-known history of NF1, 3 had a relapse history, 2 had undergone the surgical excision of more than one neurofibroma and 3 were affected by MPNSTs (one of whom developed pulmonary metastases a year later).

The group A lesions were smaller than the Group B lesions: group A mean diameter=0.84 cm (range 0.3-2 cm), 15/18 (83%) had a diameter <1 cm; group B mean diameter=3.8 cm (range 0.3-17 cm), 14/20 (70%) had diameter >1 cm ($p<0.05$, Mann-Whitney *U*-test).

CD10 expression was significantly different in the two groups: In group A 13/18 cases (73%) stained negatively and only a weak, focal CD10 positivity was present in 5/18 (27%); in group B, 19/20 cases (95%) stained positively for CD10; ($p<0.0001$, paired *t*-test).

On the contrary, the immunohistochemical assay performed with S100 and CD34 showed no statistically significant difference between the two groups: S100 stained positively in all of the group A cases and in 18/20 cases (90%) of the group B cases (the two negative ones consisting of 2/3 MPNSTs). CD34 stained positively in 17/18 (94%) of group A cases and in 15/20 cases (75%) of group B cases.

It is interesting to note that in a patient who underwent surgical removal of two neurofibromas, both were CD10 positive and in one of them CD10 highlighted just the peripheral plexiform areas that were not well evident on haematoxylin-eosin staining.

Furthermore, in another patient with primary cutaneous MPNST, there was positive staining both for S100 and CD10, nevertheless, pulmonary metastases observed one year later lacked S100 expression but stained positively with CD10, just like the primary tumor, suggesting for CD10 may be useful in the diagnosis of metastatic MPNSTs, mainly when S100 positivity has been lost.

In conclusion, CD10 proved to be able to distinguish Group A lesions from Group B lesions, with 95% sensitivity and 72% specificity; it proved negative in benign, localized neurofibromas and positive in higher risk lesions, in NF1 cases, in recurrent neurofibromas (as early as at the first removal), in MPNST and their metastases (including those negative for S100 and CD34). Moreover CD10-positive immunostaining was related to the size of the lesions and was able to highlight histological areas (myxoid, plexiform, atypical areas) with features suggesting a greater risk of malignant potential. As recently evidenced in melanoma progression, these observations could be related to the synergetic increase of *NEP* gene transcription (the gene encoding CD10 antigen), together with the co-expression of many genes involved in cell proliferation and cancerogenetic mechanisms, such as the MAP-kinase pathway, apopotosis and WNT signaling inhibition, hyper-expression of the proliferation marker Ki 67 and so on (8, 14). It is not clear, however, if CD10 hyper-expression could have a causal role in these cancerogenetic events or if it represents only an epiphenomenon of them, or if the synergetic gene over-expression observed in melanomas also applies to neurofibromas. In any case, whatever the mechanism underlying CD10 over-expression, its diagnostic and predictive value cannot be ignored.

Thus, although not specific because it is expressed in other neoplasias of different origin, prompt assessment of CD10 at the first resection, together with S100, might help in the histological diagnosis of NF1 and might allow a more correct management of higher-risk CD10-positive cases, with a follow-up aimed at limiting any serious complications of NF1 by early identification of any eventual malignant transformation.

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TARGETING THE MOLECULAR CDC37 INHIBITS MULTIPLE PROTEIN KINASES AND INHIBITS GROWTH OF PROSTATE CARCINOMA

Phillip J. Gray, JR, Thomas Prince, Jinrong Cheng, Mary Ann Stevenson and Stuart K. Calderwood

Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA

Members of the ninety kilodalton heat shock protein (HSP90) family are known to bind and stabilize intermediates in a wide variety of cell signaling pathways and contribute to their

dysregulation in cancer. An important intracellular co-factor for HSP90 is Cdc37, a protein with a broad role in fostering the activities of protein kinases. By targeting Cdc37 using RNA interference approaches, we have shown that that loss of Cdc37 function induces irreversible growth arrest in androgen receptor positive and negative prostate carcinoma cells and sensitizes such cells to chemotherapeutic drugs. In contrast to HSP90-directed agents, Cdc37 targeting appears to affect cancer cells through a distinct mechanism, and does not significantly deplete the intracellular levels of most known HSP90 client proteins. Instead, Cdc37 depletion inhibits cellular kinase activity and flux through growth promoting signal transduction cascades. We show that loss of Cdc37 leads to reduced activity of the Erk, Akt, mTOR and androgen-induced pathways. Cdc37 inactivation proved to be more effective in inhibiting prostate carcinoma growth than individual tyrosine kinases, indicating the power of multi-target therapy through Cdc37 inhibition. We have also discovered synergistic interactions between Cdc37 inactivation and the HSP90-inhibitory anticancer drug 17AAG. These interactions involve enhanced degradation of proteins essential for growth and inhibition of 17AAG-induced expression of the anti-apoptotic heat shock protein 70. Thus Cdc37 is essential for maintaining prostate tumor cell growth and may represent a novel target in the search for multi-targeted therapies based on the HSP90 chaperone system.

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SAFETY AND EFFICACY OF RADIOFREQUENCY (RF) IN LUNG TUMOURS

Nahúm Calvo Malvar[1], Lidón Millá Rallo[1], Juan Escobar Ulloa[1], Elena Rebenaque Figueras[1] and Alfredo Millá-Santos[2]

[1]CRC Corporación Sanitaria, Hospital Universitario San Joan de Reus. C/President Companys s/n. Reus (Tarragona) C.P 43201;
[2]Servicio de Oncología Clínica, Hospital Nuestra Señora del Remei Barcelona, C.P. 08024, Spain

Purpose: To determine the feasibility and safety of treating 21 lung tumours with a multiple-tine electrode switching-generator RF ablation system (Le Veen, Boston Scientifics). *Materials and Methods:* Between January 2006 and July 2008, 17 patients (mean age 68 years, range 50-80) with 21 pulmonary lesions (13 primitive non-small cell lung cancers and 8 metastases from corectal cancer) underwent the ablation procedure. All the patients had absolute contraindications to surgery. The method was performed under CT scan guidance using a expansible Le Veen needle electrode. To evaluate safety, 2 different groups were defined: i) "Normal" risk (NRG) (n=17 lesions) with adequate respiratory reserve (n=9) or insuficient respiratory reserve (n=8). ii) High risk group (HRG) (patients with domitialiry O_2-therapy n=2 patients or patients with previous lobectomy n=2). To evaluate results and complications, the SIR reporting standards for image-guided tumour ablation were followed. *Results:* 5 complications were detected (23% of the procediments) Only 2 patients in the NRG presented complications (11.7%) meanwhile complications were appreciated in 3 patients of the HRG (75%). Main complications were: NRG 1 case with dyspnea + pleural effussion, 1 case with secondary stroke; HRG 2 pneumothorax, both needing drainage and 1 infection. Treatment was completed in all the cases and no deaths were related with the procedure. A initial complete ablation was obtained in 21 of the 21 tumours (100%). Mean follow-up is 17 months (range 4-31). 85% of the patients are alive and 69% free of disease. 1 patients died due to tumour progression, and a patient died due to ARI. *Conclusion:* RF is an efficient method to treat patients with "non-surgical" lung tumours (both primary or secondary lesions). The procedure is, in general, well tolerated with a low incidence of severe complications. Good results in terms of local tumour control were received in our short-term follow up-evaluation.

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PRO AND CONS FOR LYMPH NODE DISSECTION IN OVARIAN CANCER

Oumar Camara[1] and Jalid Sehouli[2]

[1]Department Obstetrics and Gynecology; Friedrich-Schiller-University,
[2]Charité, Department of Gynecology and Obstetrics, Charité, University Medicine of Berlin, Germany

Up-front maximal surgical effort at cytoreduction with the goal of no residual disease is necessary for a better outcome. Systematic resection of para-aortic and pelvic lymph nodes is now over decades part of the surgical procedure. In early stage ovarian cancer the goal of the lymphadenectomy as a key part of surgical staging is accepted. Controversies remain on the value of systematic lymphadenectomy in patients with advanced ovarian cancer. The effect of lymph node dissection on progression free survival and overall survival in patients with advanced ovarian cancer is unknown. In patients with residual tumor, there is no indication for systematic lymph node dissection. To date there is no randomised study comparing systematic lymphadenectomy versus no lymphadenectomy in patients with no residual tumor. The Lymphadenectomy In Ovarian Neoplasms (LION) study address this Question and will be discussed. Furthermore, the techniques (*e.g.* sampling and systematic lymh node dissection), the indication as well as the pros and cons of pelvic and paraaortal lymph node dissection in early and advanced stage based on the available evidence, will be discussed.

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EXTRACELLULAR RELEASE OF HSP60 FROM TUMOR CELLS

F. Cappello[1], A.M. Merendino[1], F. Bucchieri[1], A. Ribbene[1], S. David1, C. Campanella[1], G. Burgio[2], D. Corona[2], M. Renis[3], G. Li Volti[3], E. Conway de Macario[4], A.J.L. Macario[4] and G. Zummo[1]

[1]Dipartimento di Medicina Sperimentale, Università degli Studi di Palermo;
[2]Dipartimento di Biochimica, Università degli Studi di Palermo;
[3]Dipartimento di Chimica Biologica, Chimica Medica e Biologia Molecolare, Università di Catania, Italy;
[4]University of Maryland Biotechnology Institute, Centre of Marine Biotechnology, Baltimore, MD, USA

Heat-shock proteins (Hsps) are often overexpressed during carcinogenesis and recent studies show that when expressed extracellularly, Hsps can mediate anticancer immune responses (1-3). However, the mechanisms by which Hsps are released from tumor cells into the extracellular space are not fully understood. We are investigating the pathways involved in Hsps release, including the Golgi-mediated and the exosomal and lipid-rafts pathways. For the present study, we examined NCI-H292 (mucoepidermoid carcinoma) and 16HBE (normal bronchial epithelial) cells. Both cell lines show normally low levels of apoptosis (annexin V assessment), while NCI-H292 cells present higher levels of Hsp60 and Hsp70 than 16HBE cells. Cells were therefore treated for 1 h with secretion inhibitors: Brefeldin A (BFA, 1 μg/ml), dimethylamiloride (DMA, 5 nM) or methylcyclodextrin (MCB, 1 mM). Extracellular supernatants from 50-80×10^6 treated or untreated control tumor cells were collected, dialyzed, lyophilized and resuspended in RIPA buffer; whole cell lysates were used as controls. Exosomes were separated from the surnatants by ultracentrifugation (2 h at 110,000×*g*). Released exosomes were quantified by measuring the activity of acetylcholine esterase. Hsp expressions in various cellular compartments were detected by Western blotting with anti-Hsp60 and anti-Hsp70 monoclonal antibodies. We found Hsp60 and Hsp70 [the latter is considered an exosomal marker (4)] in the exosomal fractions of the NCI-H292 cells, but only Hsp70 was detected in the 16HBE-derived exosomes. DMA and MCB but not BFA inhibited Hsp60 secretion ($p<0.05$), indicating that the preferential secretory route for this protein is through exosomes. Elucidation of the secretory pathways for Hsp60 and other Hsps is fundamental in understanding their role in antitumor immune responses and in tumor survival, as well as spread mechanisms that may represent a novel target for treatment (5).

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TWO ISOELECTRIC VARIANTS OF HSP10 ARE DOWN-REGULATED BY CIGARETTE SMOKE EXPOSURE IN AIRWAY CELLS: A PROTEOMIC STUDY

G. La Rocca[1], R. Anzalone[1], S. Corrao[1], F. Magno[1], T. Loria[1], M. Lo Iacono[1], F. Farina[1], A.M. Timperio[2], L. Zolla[2], E. Conway de Macario[3], A.J.L. Macario[3], G. Zummo[1] and F. Cappello[1]

[1]Dipartimento di Medicina Sperimentale, Università di Palermo;
[2]Departimento di Scienze Ambientali, Università della Tuscia, Viterbo, Italy;
[3]Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA

Hsp10 expression has been investigated in several cancer models, with contrasting results. It is homologue to early pregnancy factor (EPF), a secreted protein which modulates the immune response of the mother *versus* the fetus. The impact of cigarette smoke (a major risk factor for lung diseases) on Hsp10 expression by airway cells has not been characterized yet.

In this work we studied the effects of non-lethal doses of cigarette smoke extract (CSE) on the expression of Hsp60 and Hsp10 in human lung cells. Proteomics was carried out by 2D-IPG, silver stain, Western blotting, and mass-spectrometry (MS). Database searches and chaperonomics were used to identify the proteins and genes of interest.

Following CSE cell exposure as compared with unstressed cells, significant variations in Hsp10 did occur, in both lung fibroblasts and epithelial cells. In unstressed cells, three isoelectric variants of Hsp10 were found, which have not been reported for any other system, yet. After CSE exposure, only the most basic isoform was still expressed. To characterize the three variants found in unstressed cells, we performed MS analyses. Digested spots were analysed by nano-RP-HPLC-ESI-MS/MS to determine the fragments' amino acid sequences. Database searches showed that the most basic variant was human Hsp10 with 56% sequence coverage, and the other two isoforms had the same amino acid sequence, even if with a lower sequence coverage.

The data thus far indicate probably that Hsp10 protein variants are due to post-translational modifications. We recently showed the *in vivo* correlation between lung cancer development and down-regulation of Hsp10 expression (1), and

proposed a model for the antitumoral role for Hsp10, together with Hsp60 (2). The precise role of Hsp10 in carcinogenesis is still unclear. The immunosuppressive activity of EPF/Hsp10 points towards a tumor-promoting role, mediating immune evasion and apoptosis resistance. On the other hand, the *in vivo* and *in vitro* evidences obtained in human lung models suggest that different Hsp10 isoforms may mediate diverse processes and should be differentially regulated.

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HUMAN ZN(II) DEPENDENT HDACs: STRUCTURES, CATALYTIC ACTIVITY AND INHIBITION

Matthew J. Bottomley, Paola Lo Surdo, Philip Jones, Christian Steinkühler, Paola Gallinari and Andrea Carfí

IRBM *P. Angeletti,* Viale Pontina Km 30,600, I-00040, Pomezia, Rome, Italy

An intense interest in histone deacetylases (HDACs) has grown in the last few years following discoveries that aberrant epigenetics associated with cancer involve the over-recruitment of HDACs, and that over-expression of HDACs is linked with various cancers. Consequently, HDACs have become appealing targets for the development of anticancer agents. One compound, Vorinostat (suberoylanilide hydroxamic acid, SAHA), has been recently approved for the treatment of cutaneous T-cell lymphomas. More recently, HDACs inhibitors have been shown to be effective both *in vitro* and *in vivo* as anti-inflammatory agents and in the treatment of cardiac hypertrophy.

Here we describe the structure of the catalytic domain of a class IIa histone deacetylase, human HDAC4, in both inhibitor-bound and inhibitor-free states and compare it with a class I enzyme, HDAC8. The structures and accompanying biochemical data explain the intrinsic low enzymatic activity of class IIa HDACs towards acetylated lysines and provide the molecular basis for the design of class-specific HDAC inhibitors. Finally, the structure of the HDAC4 catalytic domain reveals a conformationally flexible structural zinc-binding domain conserved only in the class IIa HDACs. Biochemical and functional data suggest that this domain is critical for HDAC4 function and for its association with partner proteins in repressor complexes.

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EGFR-FAMILY EXPRESSION AND IMPLICATIONS FOR TARGETED RADIONUCLIDE THERAPY

J. Carlsson

Biomedical Radiation Sciences, Uppsala University, SE-75185, Uppsala, Sweden

High expression in the primary tumor of receptors in the EGFR-family is most often also accompanied by a similar high expression in corresponding metastases. This makes these receptors interesting as putative targets for targeted radionuclide therapy of metastases and disseminated tumor cells. The expression of all four family members, EGFR, HER2, HER3 and HER4 is reviewed in this chapter. Studies on breast, urinary bladder, colorectal, prostate, head and neck, esophageal and glioma tumors are described and possible strategies for targeted radionuclide therapy are discussed. Quantification of receptor expression and the possible influence of genomic stability on the expression are also discussed.

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EFFECTS OF LOW DOSE-RATE RADIATION ON CELLULAR SURVIVAL

J. Carlsson

Biomedical Radiation Sciences, Uppsala University, SE-75185, Uppsala, Sweden

The experience of external radiotherapy can only to a limited extent be used to understand therapeutic effects of radionuclide therapy. A major difference is that the dose-rate at radionuclide therapy is at least two orders of magnitude lower. Part of this chapter deals with estimates of the necessary dose-rate and exposure time in combination in order to deliver therapeutic effects to tumour cells. It is proposed that combinations of about 0.1-0.2 Gy/h for several days or about 1 Gy/h for at least 1 day is necessary. Such dose-rates can be achieved with the help of cross fire radiation. Effects of radionuclide therapy in terms of apoptosis, cell-cycle blocks and hyperradiosensitivity are also discussed.

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ROLE OF LESION BYPASS BY DNA POLYMERASE ETA, AND PIK KINASE SIGNALLING, IN THE RESPONSE OF HUMAN CELLS TO PLATINUM-BASED CHEMOTHERAPEUTIC AGENTS

Séverine Cruet-Hennequart, Sangamitra Villalan, Elaine O'Meara and Michael P. Carty

DNA Damage Response Laboratory, Biochemistry Department, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland

The widely-used cancer chemotherapeutic agents cisplatin, oxaliplatin and carboplatin, act primarily by induction of DNA damage, including monoadducts, intrastrand and interstrand crosslinks, leading to inhibition of DNA replication and ultimately to cell death. Targeting pathways

used by cells to overcome DNA damage is one approach to increasing the effectiveness of these anticancer agents. The capacity of cells to carry out replication of damaged DNA, using the process of translesion synthesis (TLS), is one mechanism by which cells can tolerate DNA damage. The DNA damage response (DDR) activated by damage-induced replication arrest plays a key role in determining the response of human cells to DNA damage. Activation of the DDR is mediated in part by phosphorylation of downstream substrates by the phosphatidylinositol-3-kinase-related protein kinases (PIKKs), ATM, ATR and DNA-PK.

The relationship between TLS and PIKK signalling in the response of human cell lines to cisplatin and carboplatin was investigated. Human XP30RO fibroblasts that lack the TLS enzyme DNA polymerase η (polη) as a result of a mutation in the *POLH* gene are more sensitive than normal GM00637 fibroblasts to cisplatin, oxaliplatin and carboplatin. To characterise the influence of cell cycle phase on the outcome of exposure to platinum-based drugs, polη-deficient XP30RO cells were synchronised in mitosis by treatment with nocodazole, and released for varying periods to generate cell populations in M-, G1-, or S-phase. Cells in each phase were treated with equitoxic doses of cisplatin (1.66 μM) or carboplatin (50 μM). In XP30RO cells, drug treatment led to delayed S-phase progression, and arrest in the G2 phase of the cell cycle. Resumption of cell cycle progression was delayed in cells treated with carboplatin compared to cisplatin-treated cells. In polη-deficient cells, platinum-induced DNA damage led to increased activation of the PIK kinases ATR, ATM and DNA-PK as demonstrated by increased phosphorylation of a number of protein substrates including chk1, nbs1 and replication protein A (RPA2). These phosphorylation events were mediated by ATR, ATM and DNA-PK respectively, as determined using a series of small molecule inhibitors of individual PIK kinases. To define the sequence of events in PIK kinase activation, the kinetics of phosphorylation of chk1 on serine 317, and of RPA2 on serines 4/8 were examined by Western blotting, following DNA damage by cisplatin and carboplatin. ATR-mediated phosphorylation of chk1 on serine 317 preceded DNA-PK-mediated RPA2 phosphorylation on serines 4/8 in all cases. The kinetics of RPA2 phosphorylation on serines 4/8 differed following treatment of XP30RO cells with equitoxic doses of cisplatin and carboplatin. Comparison of RPA2 phosphorylation in response to the two platinum agents with their effects on cell cycle progression indicated that RPA2 hyperphosphorylation on serines 4/8 correlated with resumption of cell cycle progression after drug-induced replication arrest in polη-deficient cells. Elucidation of the precise sequence of events that occur in response to DNA damage by cisplatin and carboplatin may help identify key DDR pathways that can be targeted to potentiate the effects of these cancer chemotherapeutic drugs.

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IMBALANCE BETWEEN SYNTHETIC LOAD AND PROTEASOMAL CAPACITY SENSITIZES NORMAL AND MALIGNANT PLASMA CELLS TO PROTEASOME INHIBITORS

Paolo Cascio[1], Giada Bianchi[2], Laura Oliva[3], Fulvia Cerruti[1], Roberto Sitia[3] and Simone Cenci[3]

[1]Department of Morphophysiology, School of Veterinary Medicine, University of Turin, *via* L. da Vinci 44, 10095 Grugliasco (TO);
[2]Department of Biology and Technology, DiBiT, San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milano, Italy;
[3]Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, MA 02115, USA

Introduction: Multiple Myeloma (MM) is an aggressive, debilitating and still incurable hematological malignancy, arising from clonal expansion of plasma cells at multiple sites in the bone marrow. Recently, MM proved sensitive to a new class of drugs, proteasome inhibitors (PI), but the mechanisms of action and bases of individual susceptibility remain still largely unclear. Obviously, their clarification would improve the clinical application of PI, and open novel therapeutic strategies. Recent work linked PI sensitivity to protein synthesis and proteasome activity (1-3), raising the question whether different levels of proteasome expression and workload underlie PI sensitivity in normal and transformed plasma cells. *Results:* To test this hypothesis we first adopted different models of mouse B cell activation *in vivo*, and observed that following polyclonal activation, proteasome activity decreases even more than previously reported *in vitro*. This decrease is linked to enhanced apoptosis after treatment with the first-in-class anti-myeloma proteasome inhibitor bortezomib (PS-341, Velcade™). Accordingly, *in vivo* treatment with bortezomib decreases Ab titres in T-dependent and -independent mouse immunization models, therefore providing the rationale for limiting the activity of Ab-secreting cells *in vivo* by impacting proteasome function. Next, to asses whether the exquisite sensitivity of certain MM cells (MMC) to PI is also due to an imbalance between limited proteasome capacity and high workload due to intense protein production, we directly assessed protein degradation by proteasomes using radioactive metabolic labeling and pulse-chase assays in two human MM cell lines – U266 and MM.1S – that display a differential apoptotic sensitivity to bortezomib. These two

lines reveal a striking direct correlation between the degradative flux of proteins through proteasomes and the apoptotic response to bortezomib. In particular, proteasomal degradation within 30 minutes of chase is almost 20 times higher in MM.1S, the far more sensitive line, indicating that these cells are intensively degrading short-lived protein species through the ubiquitin-proteasome pathway. Paradoxically, cell extracts from MM.1S cells show 2.5 times lower proteasomal activity than U266 cells, suggesting a decreased pool of proteasomes as compared to the resistant line. In this scenario, accumulation of poly-ubiquitinated proteins and the accompanying decrease of free ubiquitin reveal proteasome stress in PI-sensitive MMC. Finally, to establish cause-effect relationships, we manipulated proteasome workload, by means of ER stressors, and proteasome capacity, *via* treatment with rapidly reversible PI, achieving profound alterations of PI sensitivity. Altogether, our data demonstrate that the balance between proteasome workload and degradative capacity represents a critical determinant of apoptotic sensitivity of MMC to PI, providing both a novel predictive tool of potential prognostic value and the framework for novel combination therapies.

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HE4 – A NEW MARKER FOR OVARIAN CANCER IN ROUTINE?

M. Casova, Z. Novotny, J. Vrzalova, O. Topolcan, L. Holubec, M. Prazakova, M. Nekulova, L. Pecen, J. Finek, V. Kokes and Z. Rokyta

Teaching Hospital and Medical Faculty in Pilsen, E. Benese 13, 305 99 Plzen, Czech Republic

Introduction: HE 4 is a novel biomarker for the management of patients with a known or suspected ovarian cancer. Tumor marker CA 125 commonly used in routine has a low specificity. *Aim:* i) to validate the sensitivity and the specificity of the tumor marker HE4 for ovarian cancer in follow-up; ii) to find an optimal multiparametric combination of tumor markers for the follow up of ovarian cancer; iii) to compare the new tumor marker HE4 with CA125 used in routine. *Groups of the study:* i) pathological group: 19 women with a malignant ovarian tumor; ii) control groups: 72 women with a benign ovarian tumor, 50 women with other malignant gynecological tumors, 20 women with a gynecological benign disease (endometritis, salphingitis, *etc*.), 40 pregnant women, 20 women with non-gynecological benign diseases (renal, cardial, liver failure) which can increase values of the tumor markers CA 125.

Methods:

Parameter	Assay	Company	Units
HE 4	EIA	FUJIREBIO Diagnostics	pM
CA 125	LIA	Beckman	kIU/L
TK	REA	Immunotech	IU/L
Monototal	IRMA	IDL	IU/L
TPS	IRMA	IDL	IU/L

Results:

Marker	Cut-off	Specificity	Senzitivity	AUC
HE4	89	97	89	0.9814
CA 125	47	97	74	0.8638
TK	17	97	63	0.8559
TPS	212	97	42	0.7686
Monototal	315	97	39	0.7863

Conclusion: All measured marker values were statistically different between ovarian cancer group and the other control groups. HE4 had the highest sensitivity of 89%. Optimal cut-off for HE4 appears to be 89 ng/L. HE4 is a new prognostic tumor marker for ovarian cancer.

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MEMBRANE STEROID RECEPTORS AS POTENTIAL DIAGNOSTIC AND THERAPEUTIC TARGETS IN BREAST AND PROSTATE CANCER

Marilena Kampa, Vassiliki Pelekanou, George Notas and Elias Castanas

Laboratory of Experimental Endocrinology, University of Crete, School of Medicine, Heraklion, 7100, Greece

Rapid, non-genomic, steroid actions (RSA) have been identified since 1962. However, only in the last decade this particular mode of steroid action has been explored in depth. RSA trigger a number of very rapid effects, including ion movement, signaling cascade activation, leading to specific subsequent gene activation, cytoskeletal changes and secretion. The nature of steroid membrane binding sites remains controversary. The most compelling theories integrate the existence of either new (possibly G-protein-coupled), or classical steroid receptors, docked to the plasma membrane through post-transcriptional modifications (palmitoylation). In breast and prostate cancer, specific binding of estrogen and androgen has been identified in tumors, but not in the peritumoral non-cancerous tissue. Activation of these sites, through non-permeable steroid analogs leads to cell survival

(estrogen) or apoptosis (androgen). In this latter case, specific signaling molecules mediate actin cytoskeleton reorganization, while a potentiation of cytoskeleton acting drugs (Taxol®) is found *in vitro* and *in vivo*. Interestingly, in the same tissues, membrane-acting steroids interact with growth factors (EGF). We have also identified an interaction of these steroids with the system EPO-EPOR, promoting cell survival, through a specific signaling cascade switch. Additionally, steroids affect the transcription of EPOR gene, interacting on the EPOR promoter, suggesting a potential drawback of a concomitant EPO-aromatase inhibitor administration. The above characteristics make membrane steroid receptor agonists a new therapeutic target in breast and prostate neoplasia.

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A NEW PARADIGM IN RECEPTOR TYROSINE KINASE REGULATION AND ITS IMPLICATIONS FOR CANCER PROGRESSION

Ugo Cavallaro

IFOM-IEO Campus, Via Adamello 16, I-20139 Milan, Italy

Receptor tyrosine kinases (RTKs) are transmembrane glycoproteins that control a variety of cellular functions. The deregulated expression and/or activity of RTKs leads to different pathological conditions, with cancer being a prominent example.

The stimulation of RTK-mediated signalling is classically triggered by the binding of soluble ligands, best exemplified by polypeptide growth factors. Other membrane proteins, such as adhesion molecules, are thought to exert a regulatory function on RTK activity by modulating their localization and/or their response to ligands.

We have recently obtained evidence that instead assign a more direct role to adhesion molecules in controlling RTK function. Indeed, working on specific adhesion molecules of the immunoglobulin superfamily (Ig-CAMs), we demonstrated their activity as molecular switches for fibroblast growth factor receptor (FGFR), a prototypical RTK. On one hand, Ig-CAMs interefere with the binding of FGF to FGFR, thus repressing FGF-induced cellular response (Francavilla *et al*., 2007). On the other hand, Ig-CAMs themselves bind to and activate FGFR, emerging as novel, unconventional RTK ligands. It is intriguing that Ig-CAMs and FGF stimulate divergent signalling cascades downstream of FGFR, implying differential mechanism of FGFR activation.

Finally, our data implicate this novel interplay between adhesion molecules and FGFR in specific events associated with tumor progression, in particular in ovarian carcinoma. These findings offer a solid rationale to explore the feasibility of targeting the Ig-CAM/FGFR crosstalk as a novel approach to cancer therapy.

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ELECTROCHEMOTHERAPY OF CANINE MAST CELL TUMOURS: COMPARISON WITH SURGICAL TREATMENT

M. Cemazar[1], N. Tozon[2], A. Cör[3] and G. Sersa[1]

[1]Institute of Oncology Ljubljana, Zaloska 2, SI-1000 Ljubljana;
[2]University of Ljubljana, Veterinary Faculty, Clinic for Small Animal Medicine and Surgery, Cesta v mestni log 47, SI-1000 Ljubljana;
[3]University of Ljubljana, Faculty of Medicine, Korytkova 2, SI-1000 Ljubljana, Slovenia

Electrochemotherapy (ECT) is a novel local tumour treatment that combines administration of chemotherapeutic drugs, cisplatin or bleomycin with application of electric pulses to the tumour. It is based on the local application of short and intense electric pulses to the cells or tissues that makes the cell membrane transiently permeable and thus allow influx of foreign molecules from extracellular space into the cytoplasm. The aim of our study was to evaluate the effectiveness of electrochemotherapy (ECT) with cisplatin and to compare it with effectiveness of surgery of mast cell tumours (MCT) in dogs.

In the present retrospective study, between January 2002 and August 2004, 25 dogs of different breeds with MCT were included into the study. The dogs were divided in two treatment groups, surgery (16 dogs with 16 tumours) and those whose owners refused surgery being included in an ECT group (9 dogs with 12 tumours). ECT was performed by intratumoral injection of cisplatin (1 mg/cm^3 tumour) and 1-2 min thereafter exposed to electric pulses (8 electric pulses of 100 μs pulse duration, amplitude to electrode distance ratio 1300 V/cm and frequency 1 Hz) that were generated by an electric pulses generator (Jouan GHT 1287) and delivered through two parallel stainless steel electrodes with an inner distance between them of 7 mm. Response rate and duration of response to the treatment were evaluated and comparison between groups was made.

The clinical stages of the tumours were stage I in 4 (45%) and stage III in 5 (55%) in dogs treated by ECT, 12 (75%) dogs treated by surgery were stage I and 4 (25%) dogs were in clinical stage III. Median size of the tumours treated by surgery was 5.2 cm^3 and 2.9 cm^3 of tumours treated by ECT. ECT resulted in comparable antitumour effectiveness as surgical treatment. In 8/16 dogs that were surgically treated, tumours recurred after 0.7 to 22.5 months, while tumour recurrence was obtained only in 2/9 dogs treated by ECT (2 and 8 months).

ECT is an easy, highly effective, and well tolerated treatment of MCT. It can be an alternative treatment to surgery, especially for small nodules in which the complete response with long duration can be obtained.

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PREDICTION OF RESPONSE IN CANCER IMMUNOTHERAPY

Dainius Characiejus

Institute of Oncology, Vilnius University, Santariškių 1, LT-08660 Vilnius, Lithuania

Various parameters of the immune system have been studied in an attempt to predict clinical response to immunotherapy, mostly in renal cell carcinoma and melanoma. These parameters include profile of peripheral blood lymphocyte subsets, circulating monocytes and neutrophils, blood natural killer cell function, T-cell function, types and quantities of tumour infiltrating lymphocytes and serum autoantibodies.

Despite observed discrepancies between immunological response to therapy and clinical outcome, the following conclusions can be drawn from the literature. Firstly, *in vivo* numbers and/or proportions of cells of the immune system seem to better correlate with clinical outcome than their function *in vitro*. Secondly, types and quantities of tumour infiltrating lymphocytes may have predictive value for immunotherapy, but probably do not have an advantage over peripheral blood lymphocyte subsets. Thirdly, development of autoimmunity, as measured by the increase in serum autoantibodies, correlates with positive outcome of immunotherapy, but does not help to select patients for this type of treatment.

A biomarker that would strongly predict the response in cancer immunotherapy remains desirable. Theoretical considerations and clinical evidence suggest that the most important prerequisite of antitumour immune response is the proliferation of cytotoxic T lymphocytes. Clonal expansion of CD8+ T lymphocytes is associated with the loss of co-stimulatory molecule CD28 and acquisition of CD57 antigen. Thus, expression of CD57 antigen on CD8+ T lymphocytes may indicate the "proliferative history" of these cells. The positive relationship between CD8+CD57+ T lymphocyte expansions and survival has been observed in leukaemia and multiple myeloma. In solid tumours, this lymphocyte subset has not yet received much attention. Our recent results suggest that high risk melanoma patients with low (<23% in CD8+ subset) pre-treatment levels of CD8+CD57+ T lymphocytes may benefit from adjuvant interferon-α. Interferon-α probably acts by increasing the numbers of these lymphocytes early during treatment.

In contrast to our results with melanoma, benefit of interferon-α treatment in renal cell carcinoma is limited to patients with high (>30% in CD8+ subset) levels of CD8+CD57+ T lymphocytes. This contradiction may be explained by the fact that the expression of CD57 antigen by CD8+ T lymphocytes has been associated not only with cytotoxic potential, but also with an immunosuppressive effect. Further studies are needed to determine more precisely the predictive value of CD8+CD57+ T lymphocytes in cancer immunotherapy.

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THE CLINICAL SIGNIFICANCE OF COLLABORATION BETWEEN DISCIPLINES IN CANCER

Konstantinos Charalabopoulos

Department of Physiology, Clinical Unit, Medical Faculty, University of Ioannina, Ioannina, Greece

Recent advances in understanding malignant disease have been dominated by creation of genetic models. Many human neoplasms develop through genetic and epigenetic alterations of oncogenes and tumour suppressor genes. The accumulation of various molecular alterations is a multistep process, which leads to carcinogenesis. The most studied model of carcinogenesis is colorectal carcinoma. With the advent of endoscopic techniques, it became possible to excise lesions in all stages of colon cancer development. Pathologists have described specific morphological features for each step and molecular studies have defined the corresponding genetic alterations. This close collaboration enabled the creation of the multi-step model of carcinogenesis.

Another example is human breast cancer. In this model, each step of tumour progression correlates with one or more distinct mutations in major regulatory genes. Furthermore, recently, molecular studies have determined specific sets of genes, whose expression is correlated with survival and risk of recurrence. Again, pathologists are closely collaborating with clinicians and basic scientists in order to optimize the characterization of the disease.

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IMPLICATIONS OF INTRANUCLEAR ORDER ON DIAGNOSIS AND THERAPY OF GENETIC DISORDERS

Jyoti P. Chaudhuri, Liliana Scott, Li Song and John R. McGill

Genzyme Genetics, 4310 E. Cotton Center Blvd, Suite 120, Phoenix, AZ 85040, USA

Cytogenetic and genetic observations indicate the existence of intranuclear order (1-3). The first level of order is the genomic integrity – maternal and paternal chromosomes comprise two exclusive groups, each controlled by one of the two centrioles of a diploid cell (4). At the second level the chromosomes of a genome generally maintain some spatial order (5).

A selection of our new data from metaphase and interphase cells of specimens of cancer cases using GTG-banding and

FISH on translocations, deletions, duplications, amplifications, trisomies, polyploidies *etc* will be presented as an additional support to the aforesaid orders of chromosomes.

Profound implications of these observations on diagnosis and therapy procedures will be discussed to define the rationale of more realistic and realizable protocols of personalized medicine with further aim towards the target gene therapy.

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TNF/TRAF-2/NIK/JNK/P38 TRANSDUCTION PATHWAY AND CANCER PROSTATE PROGRESSION

B. Fraile[1], C. Chaves[1], Gonzalo Rodríguez-Berruguete[1], P. Martínez-Onsurbe[2], F. Bethecourt[3], R. Cansino[3], R. Paniagua[3] and M. Royuela[1]

[1]Department of Cell Biology and Genetics, University of Alcalá;
[2]Department of Pathology, Príncipe de Asturias Hospital. Alcalá de Henares;
[3]Department Urología del Hospital La Paz. Madrid, Spain

Aims: TNFα exerts apoptosis throughout an intracellular transduction pathway that involves the kinase proteins TRAF-2 (integration point of apoptotic and survival signals), ASK1 (pro-apoptotic protein), MEK-4 (p38 activator and metastasis suppressor gene), JNK (stress mitogen activated protein kinase) and the transcription factor AP-1. TNFα also exerts proliferation by p38 activation, or when TRAF-2 simultaneously induces the transcription factor NF-κB by NIK. NIK and p38 may also be activated by IL-1. P38 activated several transcription factors such as Elk-1, ATF-2 and NF-κB. NIK also may activate NF-κB. The aim of this study was to elucidate the possible involvement of this transduction pathway in prostate cancer development and its role in the breakdown of the apoptosis-proliferation equilibrium. *Methods:* Immunohistochemical and Western blot analyses were performed in 20 samples of normal prostates, 35 samples of BPH, and 93 samples of PC (low, medium or high Gleason grades). *Results:* 60%, 100% and 100% normal prostates showed positive immunostaining to TRAF-2, JNK, and NIK (respectively) and immunoreaction were observed in cytoplasm of epithelial cells. Immunoreaction to p38 (18%) in NP was found in the nucleus of epithelial cell. The same location was observed in PC to TRAF-2 and NIK. No immunoreaction to JNK was found in PC samples. To TRAF-2, percentages of positive patient samples decreased with the malignancy, but the immunoreaction intensity was increased in PC (no differences were found between Gleason groups). For NIK, percentages and optical density were higher in PC samples, but no differences were observed between the three Gleason groups. P-p38 was found with cytoplasmic and nuclear location in the 89% of PC samples. *Conclusion:* In PC we sugget that TNF-α/AP-1 pathway is probably inactivated by other factors such as p21 (at ASK-1 level), or bcl-2 (at JNK level) whereas proliferation and/or apoptosis inhibition pathways mediate by NIK and p38 might be activated. P38 and NIK activate different transcription factors related with cell proliferation and survival such as ATF-2, Elk-1 or NF-κB. This activation increased in patient with high Gleason score. Additional fundamental research with these different pathway members and their correlation with clinical experimentation will be required in the field of prostate cancer.

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IL-1/NIK/NF-κB TRANSDUCTION PATHWAY: A COMPARATIVE STUDY IN NORMAL AND PATHOLOGICAL HUMAN PROSTATE (BENIGN HYPERPLASIA AND CARCINOMA)

B. Fraile[1], C. Chaves[1], Gonzalo Rodríguez-Berruguete[1], P. Martínez-Onsurbe[2], G. Olmedilla[2], R. Paniagua[1] and M. Royuela[1]

[1]Department of Cell Biology and Genetics, University of Alcalá;
[2]Department of Pathology, Príncipe de Asturias Hospital. Alcalá de Henares. Madrid, Spain

Aims: It has been proposed that TNFα induces cell death, but also cell proliferation by activation of NF-κB, which may also be activated by IL-1-α. The aim of this study was investigate upstream (TRAF-6, IRAK-1) and downstream (Ikkα/β, IκBα, p-IκB, NF-κB-p50 and NF-κB-p65) components of NIK transduction pathway in normal prostate, benign prostatic hyperplasia (BPH) and prostatic carcinoma (PC). *Methods:* Immunohistochemical and Western blot analyses were performed in 20 samples of normal prostate, 35 samples of BPH, and 93 samples of PC (low, medium or high Gleason grades). *Results:* In normal prostates, cytoplasm of epithelial cells immunostained intensely to IRAK (100% of samples), TRAF-6 (80%), NIK (100%), Ikkα/β (80%), IκBα (40%) and p-IκB (60%); weakly to NF-κB-p50 (60%); and negative to NF-κB-p65. In BPH cytoplasm of epithelial cells immunostained was intensely to IRAK (99%), TRAF-6 (87%), NIK (94%), Ikkα/β (54%), IκBα (69%), p-IκB (81%); and weakly to NF-κB-p50 (100%) and NF-κB-p65 (71%). In PC, cytoplasm of epithelial cells immunostained intensely to

IRAK, TRAF-6, NIK, Ikkα/β (increased with Gleason), IκBα (increased with malignancy) and p-IκB (decreased with Gleason); and weakly to NF-κB-p50 (increased with malignancy) and NF-κB-p65 (decreased with Gleason). Nuclear inmmunostaining was only observed for NF-κB (p50 and p65), only in PC and independently of Gleason grade. *Conclusion:* We concluded that NF-κB (p50 and p65) enhances cell proliferation, but also several transcription factors, such as ATF-2 or Elk-1. In this way, several multiple transduction pathways may be involved in the uncontrolled apoptosis/cell proliferation balance. Since another study carried out on the same patients revealed that immunoexpression of proinflammatory cytokines, such as IL-1α or TNFα, increased in PC, inhibition of these cytokines might be a possible target for PC treatment, because such inhibition could decrease the activity of all transduction pathway members that activate transcription factors such as NF-κB, Elk-1 or ATF-2.

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BIOASSAY-GUIDED IDENTIFICATION AND ISOLATION OF ANTICANCER COMPONENTS IN *HYPHOLOMA FASCICULARE* EXTRACT

Wai San Kitty Pang[1], Ming Chen[2], Lin Zhai[2] and S. Brøgger Christensen[1]

[1]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen Ø; [2]Department of Clinical Microbiology, University Hospital of Copenhagen (Rigshospitalet), Tagensvej 20, DK-2200 Copenhagen N, Denmark

Previously we have found that the crude extract of *Hypholoma fasciculare* has potent *in vitro* anticancer activity against EL_4 (murine leukemia), MCF_7 (breast cancer) and PC_3 (prostate cancer) cell lines. This study is to identify and isolate active anticancer components from the mushroom.

A bioassay-guided fractionation was used to identify and purify active agents from the mushroom. Twenty different fractions were isolated from the mushroom and their *in vitro* anticancer activity was tested against EL_4, MCF_7 and PC_3 cancer cell lines with a standard high-flux anticancer-drug screening method.

Two triterpenes (fasciculol C and fasciculic acid B) were identified and isolated from the apolar fractions of the mushroom extract with IC_{50} against EL_4 at 25.5 and 19.6 μg/ml, MCF_7 at 55.5 and 40.0 μg/ml, and PC_3 at 57.0 and 60.5 μg/ml.

A polar fraction of the mushroom extract exhibits more potent *in vitro* anticancer activity than the two triterpenes with IC_{50} against EL_4 at 10.0 μg/ml, MCF7 at 15.5 μg/ml, and PC_3 at 25.3 μg/ml. The active constituents have not been identified yet.

The results illustrate that *Hypholoma fasciculare* has a potent *in vitro* anticancer activity and the active agents isolated from the mushroom could be used as a starting point for developing of anticancer agents.

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ANTICANCER ACTIVITIES OF DIFFERENT TEAS

Lin Zhai, Ming Chen and Arsalan Kharazmi

Department of Clinical Microbiology, 7602, University Hospital of Copenhagen (Rigshospitalet), Tagensvej 20, Copenhagen, DK-2200 N, Denmark

There are many reports on the anticancer activity of green tea in the recent years. In this study, we have investigated the *in vitro* anticancer activity of other teas, including 25 different Chinese teas, which belong to five different tea groups (fermentation tea, non-fermentation tea, half-fermentation tea, flower tea, and white tea), and four European teas, and one coffee.

Water extractions of the teas and coffee were made and were sterile filtered through a 0.22-μm-pore-size Millipore filter. The *in vitro* anticancer activity of these tea water extractions have been tested against three different cancer cell lines, EL_4 (leukemic cell line), MCF_7 (human breast cancer cell line), and PC_3 (human prostate cancer cell line). McCoy cell (mouse fibroblasts cell line) has been used as a non-cancer cell control. A standard assay (sulforhodamine B staining method) for anticancer-drug screening recommended by National Cancer Institute has been employed for the study.

The results show that most of tested teas exhibit potent inhibitory activity on the *in vitro* growth of the three tested cancer cell lines. The anticancer activity of non-fermentation tea, half-fermentation tea, and white tea are stronger than other teas. Among three tested Jasmine teas, one of them has much stronger anticancer activity than other two, which indicates that where tea grow and the way of tea preparation may play an important role, too. Water extraction of coffee also shows a slight inhibitory effect on EL_4 cell, but no effect on other two cancer cells. The anticancer activity of teas is significantly stronger than that of coffee.

Preliminary toxicity studies show the tested teas have no toxicity on the human mononuclear cells at the concentration 16 times higher than the IC_{50} against EL_4 cell. The *in vitro* inhibitory effect of the tested teas on a mouse fibroblasts cell line, McCoy, is also at least four times less than that on EL_4 cell.

Our data indicate that other teas have also potent anticancer activity. Since tea is a very popular beverage, further study on identifying and isolating active components from these teas and study on mechanism of action are necessary.

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CRUDE EXTRACT OF *DESMODIUM GANGETICUM* INDUCES HUMAN A549 LUNG CANCER CELL DEATH THROUGH CELL-CYCLE ARREST IN G_1 PHASE

Yuh-Fung Chen[1,2,4], Yi-Hsien Lu[1] and Huei-Yann Tsai[2,3,4]

[1]Graduate Institute of Chinese Pharmaceutical Sciences, [2]Department of Pharmacology, and [3]College of Pharmacy, China Medical University, 91 Hsueh-Shih Road, Taichung, 40402, Taiwan;
[4]Department of Pharmacy, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 40402 Taiwan, ROC

Background: Desmodium gangeticum, Leguminosae, has been widely used as a traditional herb in Taiwan and other countries. In past decades, it has been reported to have anti-inflammatory activity in carrageenan-induced inflamed rats, and to improve the severity of myocardial infarction and anti-ulcer potential in pyloric ligation and histamine induced gastric ulcer in rats and pigs. In this study, we explored its anti-nociceptive and antitumor activities in lung cancer cells. *Methods:* As an *in vivo* test, the formalin-induced nociceptive behavior was employed. *In vitro*, the growth of various various bacteria was evaluated. The MTT viability assay was used for the evaluation of growth inhibition in In lung cancer cells. Flow cytometric analysis and Western blot were used to detect the cell cycle arrest and apoptosis in *Desmodium gangeticum* treated lung cancer cells. Results: In this preliminary study, *Desmodium gangeticum* inhibited the biting and licking behavior induced by formalin in a dose-dependent manner. Additionally, it was shown that *Desmodium gangeticum* could completely inhibit the growth of Pseudomonas and partly that of *E. coli* at higher concentrations. However, it had no effective on the Klebsiella species. Additionally, *Desmodium gangeticum* dose-dependently inhibited cell viability and induced G_1 phase arrest with down-regulation of cyclin A and B1, and up-regulation of P21, P27 in lung cancer cells. *Conclusion: Desmodium gangeticum* exhibited anti-nociceptive and anti-bacterial effects. It also inhibited cell viability and induced cell cycle arrest in G_1 phase in lung cancer cells.

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UROTHELIAL ORIGIN OF CLEAR CELL ADENOCARCINOMA OF THE URINARY BLADDER

Liang Cheng[1], Ming-Tse Sung[2], Shaobo Zhang[2], Gregory T. MacLennan[3], Antonio Lopez-Beltran[4], Rodolfo Montironi[5], Mingsheng Wang[2] and Puay-Hoon Tan[6]

[1]Departments of Pathology and Urology, Indiana University School of Medicine, Indianapolis, IN, USA;
[2]Department of Pathology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan, ROC;
[3]Department of Pathology, Case Western Reserve University, Cleveland, OH, USA;
[4]Department of Pathology, Cordoba University, Cordoba, Spain;
[5]Institute of Pathological Anatomy and Histopathology, School of Medicine, Polytechnic University of the Marche Region (Ancona), United Hospitals, Ancona, Italy;
[6]Department of Pathology, Singapore General Hospital, Singapore

Clear cell adenocarcinoma in the urinary tract is an extremely rare neoplasm predominantly occurring in adult females, and morphologically identical to tumors of the same name that arise in female genital organs. Its precise histogenesis has remained controversial. We analyzed molecular genetic evaluation by fluorescence *in situ* hybridization (FISH) and X-chromosome inactivation with conventional morphological and immuno-histochemical analyses in 12 patients with clear cell adenocarcinomas in the urinary tract. Concurrent urothelial carcinoma or urothelial carcinoma *in situ* were present in 6 cases (50%) and foci of cystitis glandularis were observed in 4 cases (33%). Neither intestinal metaplasia nor Müllerian component was identified in any case. Cytoplasmic expression of α-methylacyl-CoA racemase (AMACR) was demonstrable in 10 of 12 tumors (83%). Moderate to diffuse immunostaining for CK7 was identified in all 12 tumors (100%), whereas only 3 of 12 (25%) tumors showed positive immunostaining for CK20. Focal uroplakin III staining was seen in 6 of 12 tumors (50%). In 5 cases (42%), focal to moderate CD10 immunoreactivity was observed. Immunostains for OCT4 and CDX-2 were completely negative in all tumors. In UroVysion FISH assays, all tumors displayed chromosomal alterations similar to those commonly found in urothelial carcinoma. Identical patterns of nonrandom X-chromosome inactivation in concurrent clear cell adenocarcinoma and urothelial neoplasia were identified in two informative female cases. Our data support an urothelial origin for most clear cell adenocarcinomas of the urinary tract, despite their morphologic resemblance to certain Müllerian-derived tumors of the female genital tract.

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SCHWANNOMA OF THE KIDNEY: A CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS

Liang Cheng[1], Stefano Gobbo[1,2], Jiaoti Huang[3], David J. Grignon[1], Mingsheng Wang[1], Guido Martignoni[2], Matteo Brunelli[2] and John N. Eble[1]

[1]Departments of Pathology and Laboratory Medicine, Indiana University, Indianapolis, IN, USA;
[2]Dipartimento di Patologia, Universitá di Verona, Verona, Italy;

[3]Department of Pathology, University of Rochester Medical Center, Rochester, NY, USA

Schwannomas of the kidney are rare, with only a few reported cases. We report three additional cases with immunohistochemical analysis. All three tumors were from females (aged 27, 35, and 59 years) and ranged from 4.8 to 8 cm in diameter. All of the patients underwent nephrectomy. The tumors were totally or partially encapsulated; two were in the hilum and one was centered in the renal cortex. All tumors were diffusely positive for S-100 protein. Two were positive for neuron-specific enolase. Immunostaining for neurofilament, HMB45, microphthalmia transcription factor, smooth muscle actin, CD34, cytokeratin AE1/3, cytokeratin 7 and CD10 were negative. Follow-up data were available for two patients; neither had tumor recurrence or metastasis. In conclusion, renal schwannoma is rare, usually arises centrally, impinging on the hilum or the pelvis and is cured by resection. Sarcomatoid carcinoma and other spindle cell tumors should be considered in the differential diagnosis.

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RENAL CELL CARCINOMAS WITH PAPILLARY ARCHITECTURE AND CLEAR CELL COMPONENTS: DIAGNOSTIC UTILITY OF CYTOGENETICAL ANALYSES

Liang Cheng[1], Stefano Gobbo[1,4], Gregory T. MacLennan[2], David J. Grignon[1], Rajal B. Shah[3], Shaobo Zhang[1], Guido Martignoni[4], Matteo Brunelli4 and John N. Eble[1]

[1]Departments of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana;
[2]Departments of Pathology and Laboratory Medicine, Case Western Reserve University, Cleveland, Ohio;
[3]Departments of Pathology and Laboratory Medicine, University of Michigan, Ann Arbor, MI, USA;
[4]Dipartimento di Patologia, Universitá di Verona, Verona, Italy

Although histological features enable an accurate diagnosis in most renal carcinomas, overlapping morphologic findings between some renal neoplasms make subclassification difficult. Some renal carcinomas show papillary architecture but are composed extensively of cells with clear cytoplasm, and it is unclear whether they should be classified as clear cell renal cell carcinomas or papillary renal cell carcinomas. We analysed the immunohistochemical profiles and the cytogenetic patterns of fourteen renal carcinomas showing papillary architecture in which there were variable amounts of cells with clear cytoplasm. The patients were eight women and six men (mean age: 54 years). Immunohistochemistry and fluorescence *in situ* hybridization analysis distinguished two different groups. The first consisted of ten renal cell carcinomas with strong immunoreactivity for alpha-methyl CoA racemase (AMACR), of which 9 also expressed cytokeratin 7. All of these neoplasms showed gains of chromosome 7 or 17 and chromosome Y was lost in all the male patients whereas 3p deletion was detected only in one case. In the other four renal cell carcinomas, cytokeratin 7 was not detected and AMACR was positive in only one. In these neoplasms, no gains of chromosomes 7 or 17 and no loss of chromosome Y were observed whereas 3p deletion was detected in three of them. None of the 14 neoplasms showed immunoreactivity for TFE3. The combined use of immunohistochemistry and cytogenetics enabled us to provide a definitive diagnosis for 12 of 14 renal cell carcinomas with papillary architecture and clear cell components: 9 cases were confirmed to be papillary renal cell carcinomas and 3 cases were confirmed to be clear cell renal cell carcinomas. Despite these ancillary techniques, two cases remained unclassified. Our study establishes the utility of these procedures in accurately classifying the great majority of renal cell carcinomas with these findings.

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THE UROVYSION FISH ANALYSIS OFFERS A PROMISING SURVEILLANCE STRATEGY IN PATIENTS WHO UNDERWENT AUGMENTATION CYSTOPLASTY

Liang Cheng[2,3], Ming-Tse Sung[1], Shaobo Zhang[2], Mingsheng Wang[2], Michael O. Koch[3], Mark P. Cain[3] and Richard C. Rink[3]

[1]Department of Pathology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan, ROC; [2]Departments of Pathology and Laboratory Medicine and [3]Urology, Indiana University School of Medicine, Indianapolis, IN, USA

Patients who have undergone intestinal augmentation cystoplasty are at risk for developing latent vesical malignancy. The present study was conducted to evaluate the histological and immunohistochemical characteristics and molecular genetic alterations in these neoplasms.

Four patients developing urothelial neoplams after augmentation cystoplasty were included in the current study. Tumor specimens were assessed for morphological features and immunohistochemical expression of uroplakin III, CDX2, β-catenin, and cytokeratin 7 and 20. Gene mutations in fibroblast growth factor receptor 3 (FGFR3) gene and p53 gene were analyzed and the UroVysion fluorescence *in situ* hybridization (FISH) tests were performed. The mean age of the patients, including two men and two women, was 37. The latency from bladder augmentation to developing malignancy

ranged from 17 to 21 years (mean 19 years). All patients died of widespread metastasis months after cancer diagnosis (mean, 5 months). In the morphological evaluation, all tumors were high-grade (grade 3) invasive urothelial carcinoma comprising various architectural patterns with brisk mitoses and tumor necrosis. Three harbored glandular differentiation (75%) and the remaining one showed squamous differentiation (25%). All cases revealed abnormal decreasing β-catenin expression with moderate to strong membranous staining in 30~60% of tumor cells. Two tumors (50%) showed nuclear expression of CDX2, with variable staining intensity and percentages. Moderate uroplakin III staining was focally identified in one case. All but one tumors (75%) were intensely stained by cytokeratin 7. One case (25%) displayed focal cytokeratin 20 expression. In UroVysion FISH analysis, all tumors displayed characteristic chromosomal abnormalities (100%). Point mutations of both FGFR3 and p53 genes were identified in one case. In summary, neoplasms developed after augmentation cystoplasty were extremely aggressive urothelial carcinomas and exhibited distinct morphologic, immunohistochemical and genetic characteristics. These neoplasms represent a rare and specific variant of urothelial carcinoma which is uniformly lethal. The UroVysion FISH analysis may offer an attractive surveillance strategy in patients who underwent augmentation cystoplasty.

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SENSITIZATION OF LIVER CANCER CELLS TO CHEMOAGENTS THROUGH GEP-TARGETED THERAPY

Siu Tim Cheung

Department of Surgery, The University of Hong Kong

Primary liver cancer, hepatocellular carcinoma (HCC), is the fifth most common cancer and the third cancer killer in the world, with about half a million individuals dying from HCC annually. The disease is frequently diagnosed at an advanced stage and thus precludes curative surgical treatment. Chemotherapy has shown marginal efficacy and is accompanied with severe side-effects. A new therapeutic strategy is essential to sensitize cancer cells to chemotoxic agents.

Granulin-epithelin precursor (GEP) was shown to be a therapeutic target for HCC treatment in our earlier studies (1). We firstly identified GEP overexpression through genomic expression profiling studies using the microarray approach (2-3). The GEP expression at the mRNA level (2-3) was subsequently validated at the protein level in more than 200 HCCs and liver tissue adjacent to tumor samples, and confirmed that more than 70% of HCC tissues revealed GEP overexpression (4-5). We showed that GEP controls HCC cell proliferation, invasion and tumorigenicity (4). These biological roles correspond to the clinical findings that expression of GEP is associated with aggressive cancer features including large tumors, venous infiltration (micrometastasis) and early recurrence after curative surgery (4). We then examined the therapeutic potential of GEP-targeted therapy using the in-house made anti-GEP monoclonal antibody (mAb) A23. The GEP mAb inhibited the growth of human hepatoma cells Hep3B and HepG2, but revealed no significant effect on normal liver cells MIHA. In a nude mice model transplanted with human HCC cells Hep3B, GEP mAb decreased the serum GEP level and inhibited the growth of established tumors in a dose-dependent manner (1). There are reports showing that GEP mediates drug resistance in some cancer types (6-8). We therefore hypothesize that GEP targeted therapy will enhance the sensitivity of HCC cells to chemotoxic agents.

We investigated the effect of GEP neutralization on the chemosensitivity of HCC cells. The biological responses of GEP mAb A23, alone and in combination with cisplatin, on HCC cells Hep3B *in vitro* and *in vivo* have been examined. Hep3B cells treated with GEP mAb A23 in combination with cisplatin demonstrated synergistic effect on induction of cell apoptosis (25.1%) compared to cisplatin alone (10.0%), GEP mAb A23 alone (0.1%) and control treatment (0%) by flow cytometry analysis. The combination therapy approach was then examined in *in vivo* system with the Hep3B xenograft in nude mouse model. We demonstrated that GEP mAb plus cisplatin can further inhibit the tumor growth (74.7%) when compared to cisplatin alone (53.8%), GEP mAb A23 alone (45.4%) with the control treatment.

In summary, the current data indicated that the GEP targeted therapy in combination with cisplatin demonstrated synergistic cytotoxic effect. The cell apoptotic event induced by cisplatin was further amplified by neutralization of GEP, and the combination treatment approach further enhanced the growth inhibitory effect of tumor xenograft in nude mice model. The mechanism of GEP-targeted therapy in sensitizing HCC cells to chemodrugs is currently underway.

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DMXAA: A VASCULAR-DISRUPTING AGENT WITH CYTOKINE/IMMUNE MODULATORY ACTIVITY

Lai-Ming Ching

Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ, USA

DMXAA, the first in its class of anticancer/vascular disrupting agents, is currently in Phase III clinical trials. We previously established that DMXAA has two distinct activities that appear critical to its potent antitumour effects in mice: the induction of tumour vascular endothelial apoptosis, followed by the production of cytokines within the tumour. Multiplex assays were used to screen for cytokines produced by human blood leucocytes (HBLs) cultured with DMXAA, with the aim of identifying a select panel of cytokines that could be used as surrogate markers of response. HBLs were cultured with DMXAA for 16 h, and the supernatants assayed using a 42-plex human cytokine kit and the Luminex 100™ MAP platform. While quantitative differences were observed between donors, a consistent pattern of modulation of a panel of 7 cytokines could be observed amongst the donors tested thus far. DMXAA inhibited constitutively-produced IP-10, MCP-1 and sCD40L, but increased the production of TNF, MIP1-α, IL-6 and IL-8 in 50% of the donors, who were designated as high responders. Our data suggest that the pattern of modulation of this panel of cytokines may be used to select for patients who will best respond to DMXAA treatment. Inclusion of such multiplex cytokine assays in the clinical trials, we suggest would also be useful in providing surrogate markers of response.

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EP2 RECEPTOR-MEDIATED ACTIVATION OF EXTRACELLULAR SIGNAL-REGULATED KINASE SIGNALING IS REQUIRED FOR THE MITOGENIC ACTION OF PROSTAGLANDIN E_2 IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

C.H. Cho and L. Yu

Department of Pharmacology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

The use of non-steroidal anti-inflammatory drugs is associated with a lower risk for esophageal squamous cell carcinoma, in which overexpression of cyclooxygenase-2 (COX-2) is frequently reported. Prostaglandin E_2 (PGE_2), a COX-2-derived eicosanoid, is implicated in the promotion of cancer growth. The precise role of PGE_2 in the disease development of esophageal squamous cell carcinoma, however, remains elusive. In this study, we investigated the effect of PGE_2 on the proliferation of cultured esophageal squamous cell carcinoma cells (HKESC-1). Results showed that HKESC-1 cells expressed all four PGE_2 receptors, namely EP1 to EP4 receptors. In this regard, PGE_2 and the EP2 receptor agonist butaprost markedly increased HKESC-1 cell proliferation. Moreover, the mitogenic effect of PGE_2 was significantly attenuated by RNA interference-mediated knockdown of EP2 receptor, indicating that this receptor mediated the mitogenic effect of PGE_2. In this connection, PGE_2 and butaprost induced phosphorylation of extracellular signal-regulated kinases-1/2 (Erk1/2), whose down-regulation by RNA interference significantly attenuated PGE_2-induced cell proliferation. In addition, PGE_2 and butaprost increased c-Myc and c-Fos expression and activator protein-1 (AP-1) transcriptional activity, which were abolished by the mitogen-activated protein kinase/ERK kinase (MEK) inhibitor U0126. AP-1 binding inhibitor curcumin and RNA interference-mediated knockdown of c-Myc also partially reversed the mitogenic effect of PGE_2. Taken together, these data demonstrate for the first time that EP2 receptor mediates the mitogenic effect of PGE_2 in esophageal squamous cell carcinoma *via* activation Erk/AP-1 and c-Myc pathway. This study supports the growth-promoting action of PGE_2 in esophageal squamous cell carcinoma and the potential application of EP2 receptor antagonists in the treatment of this disease.

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CANCER BIOMARKER DISCOVERY BY ONCOPROTEOMICS

William C.S. Cho

Department of Clinical Oncology, Queen Elizabeth Hospital, Room 1305, 13/F, Block R, 30 Gascoigne Road, Kowloon, Hong Kong

Cancer is the leading cause of death in most of the developed countries. Early diagnosis and prevention are key factors needed to reduce the mortality and morbidity of all types of cancer. Traditional diagnostic methodologies, to a certain extent, have their limitations of sensitivity and specificity. It is imperative that more useful and specific cancer biomarkers are discovered.

Proteomics is the large-scale study of proteins. With the advent of new and improved proteomics technologies, such as the development of quantitative proteomics methods, high-resolution, -speed and -sensitivity mass spectrometry and protein arrays, as well as advanced bioinformatics for data handling and interpretation, it is now possible to discover biomarkers that can reliably and accurately predict outcomes during cancer management and treatment.

Oncoproteomics is the study of proteins and their interactions in a cancer cell by proteomics technologies. By studying the interrelationships of protein expressions and modifications in cancer and normal cells, proteomics contributes important insights into the pathophysiological basis of cancer. Oncoproteomics has the potential to be applicable for clinical practice, including cancer diagnosis and screening based on proteomics platforms as a complement to histopathology, individualized selection of therapeutic combinations that target the entire cancer-specific protein network, assessment of therapeutic efficacy and toxicity, and rational modulation of therapy based on changes in the cancer protein network associated with prognosis and drug resistance. In addition, protein biomarkers identified can also serve as therapeutic targets and provide mechanistic approach for the study of drug effects as well as effective drug design.

In this presentation, some remarkable discoveries of cancer biomarkers as well as the challenges ahead and perspectives of oncoproteomics for biomarker development will be overviewed.

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EMODIN-INDUCED MULTIPLE DRUG-RESISTANCE GENE EXPRESSION IN RAT C6 GLIOMA CELLS THROUGH AFFECTING MAPK SURVIVAL AND NF-KAPPAB SIGNALING PATHWAYS

Jing-Gung Chung[1,2,3]

[1]Departments of Microbiology, [2]Pharmacology, [3]Biological Science and Technology, China Medical University, Taichung, Taiwan, ROC

We wanted to test whether or not emodin has suppressive effects on glioma cells. In the present study, emodin inhibited the proliferation and induced apoptosis of C6 cells at a 12 h treatment, but surprising results showed that C6 cells survived a 72 h drug treatment and resistance to emodin occurred. C6 cells overcame emodin-induced apoptosis by inhibition of the expression and activation of apoptosis-associated proteins including p53, Bax, Bcl-2, Fas and caspase-3. C6 cells were able to express antioxidant proteins (SOD and catalase) to reduce ROS-induced cytotoxity of emodin and overexpress multi-drug resistance genes (*Mdr1a, MRP2, MRP3* and *MRP6*) to decrease the intracellular accumulation of emodin. EMSA analysis showed that emodin decreased NF-κB expression at 24-h treatment but at 48 h treatment, emodin increased NF-κB activity. Confocal microscopy showed that emodin induced NF-κB translocation from cytoplasma to nuclei. C6 cells activated the MAPK survival pathway and expressed DNA repair gene (*MGMT*) and associated proteins (*PARP* and *XRCC1*) to recover cell activity. C6 cells also expressed GRP78 to decrease emodin-induced ER stress which would cause apoptosis in C6 cells, and GRP78 inhibited the expression of GADD153 to enhance the expression of Bcl-2 which could balance the ER-induced and mitochondria-induced apoptosis of C6 cells.

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THE ROLE OF PROTEIN PHOSPORYLATION IN BREAST CANCER

Jonas Cicenas

Evolutionary Biology, Zoological Institute, University of Basel, Vesalgasse 1, CH-4051, Basel, Switzerland

Molecular analysis of cancer tissue samples, carried out for diagnostic and prognostic purposes, provides valuable data about the expression of individual proteins or genes. The most frequent method for extracting molecular information from human tissues is to visualize protein levels in tissue either by immunohistochemistry or enzyme immunoassay approaches. Such analysis, however, remains limited in its ability to elucidate the function of such proteins. Protein function is regulated not only by transcription and translation, but also by such posttranslational modifications. On of the most important of these modifications is phosphorylation, which lies behind most pathways cell signal transduction and is thus fundamental both normal physiology and disease. Given these considerations, the number of proteins in the activated (phosphorylated) state may be more important biologically than the total number of proteins present. Our data suggests that protein phosphorylation, provides either additional or independent prognostic value for primary breast cancer patients.

In this overview we will demonstrate, that increased levels of phosphorylated akt predicts poor prognosis in breast cancer.

We will also discuss some data showing, that phosphorylation of ErbB-2 is an independent predictor of poor prognosis in primary breast cancer patients. In addition we will show some new, unexpected and yet unpublished results on SchA phopsorylation in breast cancer.

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INFLUENCE OF THE STRUCTURE OF NEW ANTHRACYCLINE ANTIBIOTICS IN THEIR STABILITY IN COMMONLY USED *I.V.* INFUSION SOLUTIONS

Judyta Cielecka-Piontek[1], Anna Jelińska[1], Agnieszka Dołhań[1], Irena Oszczapowicz[2] and Małgorzata Wąsowska[2]

[1]Poznan University of Medical Sciences, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Poznań; [2]Department of Modified Antibiotics, Institute of Biotechnology and Antibiotics, Warsaw, Poland

The aim of this study was to determine the influence of the structure of new derivatives of daunorubicin, which were synthesized at the Institute of Biotechnology and Antibiotics in Warsaw (Poland) (1), on their stability in various commonly used *i.v.* infusions solutions. This study is a continuation of biological activity investigations of these derivatives (2, 3).

The stability of daunorubicin derivatives containing at position C-3' daunosamine a piperidine (DD–1), morpholine (DD–2), pyrrolidine (DD–3) or hexahydroazepine (DD–4) moiety was studied in aqua pro injectione, 0.9% sodium chloride, 5%, 10%, 20% glucose, Ringer's solution, lactated Ringer's injection, mixture of 0.9% sodium chloride and glucose (1:1; 1:2), pediatric solution, multielectrolic solution, Jonosteril®Basic solution and 20% mannitol after store: at room temperature (2, 4, 6, 24 h), at 2-8˚C (2, 6 24 h) and at –16˚C (30 days).

The derivatives were recognized as stable when changes in their initial concentration did not exceed 10%. After storage at room temperature the derivative with pyrrolidine moiety was the most stable (in 84.6% of the solutions) whereas the morpholine derivative was stable only in two of the solutions during 2 h.

After storage at 2-8˚C stability of DD–1 in seven solutions, DD–2 in three solutions, DD–3 in four solutions and DD–4 in nine solutions were bigger. However, storage of DD–3 solutions in aqua pro injection, 0.9% sodium chloride and DD–4 in multielectrolic, Jonosteril®Basic and 5% glucose solutions at –16˚C 30 days decreased their stability.

A previous study showed that the derivative with morpholine moiety had the highest biological activity, whereas it was the less stable in *i.v.* infusions solutions therefore solutions of this derivative have been prepared *ex tempore*.

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THE CHICK EMBRYO CHORIOALLANTOIC MEMBRANE AS AN *IN VIVO* MODEL FOR STUDY TUMOR ANGIOGENESIS AND METASTASIS

Anca Maria Cimpean[1], Domenico Ribatti[2] and Marius Raica[1]

[1]"Victor Babes" University of Medicine and Pharmacy Timisoara, Department of Histology, Piata Eftimie Murgu, nr. 2, 300041, Timisoara, Timis, Romania; [2]University of Bari Medical School, Department of Human Anatomy and Histology, Piazza G. Cesare, 11, Policlinico, 70124 Bari, Italy

Tumor angiogenesis and metastasis – two sides of the same coin, two processes which are directly involved in the progression of malignant tumors. Many unresolved problems concerning these two aspects impair the clinical management of the tumors and also the choice of proper therapy. Most heterologous transplants of human and rodents tumors can survive on chick CAM by several passages with different patterns, and the intravascular and extravascular pathways of tumor metastasis can be easily differentiated on CAM model. The behaviour of a tumor and its metastasis are different concerning therapy response and prognosis because of an incomplete molecular characterization of the tumors and their microcirculation. Metastases and their vascularization are not well understood. The chick embryo chorioallantoic membrane (CAM) represents an experimental model for dynamic study of tumor metastasis and angiogenesis. The rich vascular network of the CAM provides a useful tool for studying the recruitment of the neovessels by the tumors implanted on the CAM. The daily direct observation of macroscopic changes of a tumor and its vascular network represents an advantage for this *in vivo* model. Many antiangiogenic agents were studied using the CAM model. Fluorescent labelled tumor cells can be directly monitored for their capability of local invasion and metastasis on chick CAM. The lack of a inflamatory response which characterizes this model in early-stages of embryonic development can help us to study tumor angiogenesis and metastasis in the absence of this process. Morphological changes and molecular characterization of metastatic cells

are little studied and could be a starting point for a better understanding of differences in the behaviour of a primary tumor and its metastasis in different organs. The reduced number of antibodies and reagents available with specificity for chick CAM blood and lymphatic vessels limits the fine characterization of the angiogenic response by using this model, but the relatively recent complete characterisation of the chick embryo genome will be helpful to synthesize a broad panel of antibodies with high specificity for chicken tissues, especially for blood and lymphatic endothelial cells and stroma components. This aspect could be useful to better characterize the interactions between implanted human and/or mouse tumors and chicken tissues. Embryonic microenvironments have been shown to inhibit the tumorigenicity of a variety of cancer cell lines. In this context, the embryonic microenvironment of the chick CAM could provide an opportunity to study the influence of such microenvironment on cancer cell development and their metastatic properties.

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PROTEOMIC ANALYSIS OF PROSTATE TUMOR TISSUE AND PROXIMAL FLUID FOR CANCER BIOMARKER DISCOVER

Brian L. Hood[1,2], Joel B. Nelson[3] and Thomas P. Conrads[1,2]

[1]Department of Pharmacology and Chemical Biology, [2]Clinical Proteomics Facility, University of Pittsburgh Cancer Institute, [3]Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Armed with sequence information of the human and mouse genomes, a major aim of biological science is toward unraveling the underlying molecular events that lead to cellular function/dysfunction in cancer, with the goal of discovering better diagnostic markers and therapeutic targets. Proteomics aims to facilitate this process by applying newly developed methods and advanced analytical tools, such as mass spectrometry, for the investigation of the protein complement *en masse*. Conventional protein biomarker discovery investigations are predominantly performed with samples such as serum/plasma. While serum/plasma is more desirable from a clinical standpoint, tissue likely possesses a greater abundance of readily identifiable proteins directly reflective of disease; however, most of these proteins are unlikely to be released from the tissue into the circulatory system, thereby limiting their clinical utility. We propose that investigation of proximal fluids may provide a novel connection between tissue and serum to permit the identification of proteins that posses a high likelihood of being directly related to pathophysiology and that are readily assayable from serum. This lecture will highlight our efforts in applying advanced proteomic discovery methodologies in identification and validation of prostate cancer biomarkers from prostate tissue and expressed prostatic secretions, a novel proximal fluid for proteomic discovery.

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PET IN BREAST CANCER

A. Constantinidou

The Royal Marsden Hospital, London, UK

Background: Positron Emission Tomography (PET) has been an exciting development in recent years, providing accurate functional information on disease status as well as anatomical information when combined with Computed Tomography (CT). The role of PET/CT in breast cancer is not yet clearly defined. *Aim:* To summarize the current data on the utility of PET in breast cancer and to assess its contribution in optimising management. *Methods:* Available data on the role of PET in breast cancer come from rather small predominately retrospective studies. *Results:* The principal areas where PET/CT appears useful in breast cancer management have been identified. It is currently the most accurate modality to define/ restage metastatic breast cancer. It is often revealing unsuspected metastases in up to 30% of patients. It is particularly useful in detecting bony lytic metastatic disease and the entity of "bone scan negative, PET/CT positive" disease is now clearly recognised. PET/CT is effective in assessing response to chemotherapy and hormonal treatment earlier than any other method currently available. It has successfully been used in the evaluation of indeterminate lesions on conventional imaging. *Conclusion:* As PET/CT becomes more widely available it is likely to be used early and extensively in the management of breast cancer. Evidence suggests it can play a key role in a number of areas. Formal guidelines for the use of this modality in breast cancer are warranted.

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SMALL RENAL TUMORS: ALTERNATIVE NON SURGICAL TREATMENTS

G. Conti

Department of Urology, St. Anna Hospital, Como, Italy

In the last two decades surgical conservative approach has become an accepted standard treatment in patients with small renal tumours either in presence of a normal contralateral kidney or in case of a solitary one or if multiple neoplastic lesions are present. More recently the development of new technologies as cryotherapy, radiofrequency and high intensity focused ultrasounds has offered an alternative way to conservatively manage small renal masses. Cryoablation is the most diffuse and

experimented technique. By a creation of a well controlled ice-ball cellular damage and necrosis are obtained (immediately related to ice crystals formation inside the sphere, late by coagulative necrosis, apoptosis regulation and vascular damage). Real time step by step control may be obtained by sonography. The preferred way is the laparoscopic one but percutaneous approach is nowadays available with the new generation's probes. Radiofrequency induces a thermal damage by the insertion of needle probes (single, multiple or spiral shaped) inside the tumour delivering a high frequency (300-800 kHz), high powered (90-200 W) electrical current that drives the temperature up to 105˚C with a non reversible coagulative necrosis. The real time control by sonography is possible but less accurate. Percutaneous approach is easily feasible with this technique. In a series presented by the Cleveland Clinic Team local failure was 1.8% for Cryo and 11.1% for radiofrequency without any difference in renal function preservation. Cancer specific survival range between 97% and 100% at a median follow-up of 3 years for both treatments in four different series of patients, and overall survival between 80% and 100% at 3 years. Tumour recurrence is 24.3% after Radiofrequency and 9.0% after Cryo 70% of recurrences is diagnosed after 3-4 months of follow-up and 92% at 1 year. Bleeding and perirenal haematoma as well as capsular fractures are the most common cryo-related morbidities; upper urinary tract damage and UPJ obstruction are more frequent after radiofrequency procedures. Post Cryo fibrosis appeared more extensive than that following primary radiofrequency when salvage surgery was performed. The principal indication for alternative techniques is very similar to that for nephron sparing surgery regarding tumours dimension, presence of solitary kidney, multiple bilateral lesions; Cryoablation and radiofrequency are more simple than surgery, morbidity is significantly decreased and, when percutaneously performed, hospital stay is very short (day or one-day surgery procedures) with comparable results in terms of cancer specific and overall survival in particular with last generation's Cryo probes.

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SYNTHESIS AND EVALUATION OF IMIDO-SUBSTITUTED-NAPHTHOQUINONE DERIVATIVES AS INHIBITOR OF MAPK SIGNALING CASCADE IN PROSTATE CANCER CELLS

Robert L. Copeland Jr.[1], Jharna R. Das[1], Oladapo Bakare[2], YaYin Fang[3], Desta Beyene[4] and Yasmine M. Kanaan[3]

Departments of [1]Pharmacology, [2]Chemistry, [3]Biochemisty and [4]Microbiology College of Medicine and Cancer Center, Howard University, Washington, D.C. 20059, USA

Prostate cancer (PC) is the most commonly diagnosed cancer in American men with an estimated 186,320 new cases diagnosed in 2008. Androgen ablation is highly effective palliative therapy; however, most men eventually relapse due to the presence of androgen independent cancer cells. Currently there are no therapies that effectively eliminate these androgen-independent PC cells. It has been reported that MAPK pathways play a critical role for survival and proliferation of androgen-independent cells. Our hypothesis is that enhanced response in PC therapy may require inhibitors that affect multiple signal transduction pathways. In our studies, imido-substituted chloronaphthoquinones have been synthesized that are selective inhibitors of MAPK. Experiments showed that 2-chloro-3-(*N*-succinimidyl)-1,4-naphthoquinone and analogs have IC_{50}s against purified MEK in the range of 0.4-10 μM. Subsequently these imido-substituted chloronaphthoquinone analogs were tested in cell proliferation assays in androgen-sensitive (LNCaP, CWR-22), androgen-insensitive (PC-3, DU-145) cells, normal bone marrow cells, HS5 and normal prostate cells, RWPE1. Growth inhibition of each cell line revealed significant antitumor activities within concentration ranges of 1-3 μM for the cancer cells. The effect of 2,3-dichloro-5,8-dimethoxy-1,4-naphthoquinone (DCDMNQ) on LNCaP, CWR22, PC3, DU145 HS5 and RWPE1 cells revealed significant antitumor activities with IC_{50}s, of 1±0.1, 3±0.3, 1.5±0.1, 3±0.3, 10±0.5 and 15±0.6 μM respectively.

On the basis of this cell-based cytotoxicity screen, DCDMNQ demonstrated the best efficacy. Cell cycle analysis

showed that DCDMNQ inhibited progression through the cell cycle in PC-3 and DU-145 cell lines in a time-dependent manner stopping at the S-phase. The result for LNCaP cell line was inconsistent; whereas, in CWR-22 cell line the drug arrested cells in G_1 phase of cell cycle with greatest proportion of cells in G_1 phase by day 5. The compound showed no effect on the cell cycle progression in bone marrow HS-5 cell line. In addition, DCDMNQ induced apoptosis in the androgen-independent cells preferentially over that of the androgen-dependent cell lines in a time-dependent manner.

These findings were further validated by Western blot analysis. To verify that DCDMNQ maintains ability to inhibit MAP kinases, Western blot analysis was performed to evaluate the effect of this compound on generation of phosphorylated MAPK protein in PC-3 and DU-145 cell lines. The results revealed that the initial insult of DCDMNQ decreased AKT activity and at later time points inhibited other cell survival pathways such as MEK 1/2, ERK 1/2, and JNK 1/2 in androgen-independent cell lines (PC3 and DU145), whereas androgen-dependent LNCap showed significant decrease in ERK 1/2 and AKT in time-dependent manner but MEK 1/2, and JNK 1/2 showed activation at 3 days following significant decrease at 5 days exposure. It was demonstrated that DCDMNQ can inhibit MEK, ERK, AKT and p38 phosphorylation either through inhibition of multiple kinases or *via* direct inhibition which would then prevent downstream phosphorylation.

Furthermore, active small molecule kinase inhibitors to date are ATP mimics that bind to the intracellular ATP-binding domain within the kinase. It has been proposed that MEK1/2 can be inhibited through a novel, noncompetitive mechanism. X-ray crystallography structure of MEK1/2 in complex with MgATP reveals that the enzyme has a unique inhibitor binding pocket adjacent to the ATP binding site. We carried out molecular modeling using DCDMNQ in concurrence with the published structure of the complex of MEK with MgATP, and the MEK inhibitor 5-bromo-*N*-(2,3-dihydroxypropoxy)-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide (BBM) to explore whether imido-substituted 1,4-naphthoquinones might bind *via* a similar non-competitive mechanism. Our studies showed that in the crystal structure a binding pocket for DCDMNQ is deep inside of MEK. Evaluation of hydrogen bonding for DCDMNQ when docked to this pocket demonstrates that it can form hydrogen bonds with LYS97, SER212 and water (HOH97). Thus the presence of DCDMNQ induces conformational changes in the unphosphorylated MEK1/2 that locks it into a closed but catalytically inactive species.

Thus cytotoxicity of DCDMNQ is mediated *via* inhibition of MAPK/AKT pathways in prostate cancer cells *in vitro*. Therefore, this compound represents a novel class of compounds which might lead to future therapeutic interventions of prostate cancer while protecting bone marrow and normal prostate epithelial cells.

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EXPRESSION OF VITAMIN D-24-HYDROXYLASE (CYP24) IN BENIGN AND MALIGNANT BREAST TISSUES AND CELL LINES

T. Cordes[1], M. Friedrich[2], S. Becker[1], K. Diedrich[1], D. Fischer[1] and M. Thill[1]

[1]Klinik für Frauenheilkunde und Geburtshilfe, UKSH Campus Lübeck;
[2]Klinik für Frauenheilkunde und Geburtshilfe, Helios Klinikum Krefeld, Germany

Introduction: It is well known that vitamin D and its metabolites have a protective effect against cancers, 1,25-Dihydroxyvitamin D_3, the biological active metabolite of Vitamin D, is known to regulate cell proliferation in various cell lines. It is also essential for the regulation of calcium and phosphate levels and of the bone metabolism. Vitamin D_3 is a secosteroid hormone which derives from 7-dehydrocholesterole. In the skin it is synthesized in combination with UV-radiation from the precursors 7-dehydrocholesterole and provitamine D_3. Hepatocytes transform it to 25-hydroxyvitamin D_3. Six cytochrome P450 hydroxylases can exhibit this 25-hydroxylation, with the main enzyme being CYP27A1 (25-hydroxylase). The subsequent step is a 1-hydroxylation by CYP27B1 (1α-hydroxylase) which produces the most active form of vitamin D_3, 1,25-dihydroxyvitamin-D_3 (calcitriol). This metabolite is inactivated by a 24-hydroxylation by CYP24 40 (24-hydroxylase). Alternative splicing frequently occurs in breast cancer cells; different splice variants of a given protein can display different biological functions and may cause tissue-specific variations. In this study we describe the expression of 24-OHase in human benign and malign breast tissue. *Methods:* Expression of 24-OHase RNA and protein was assessed by real-time-polymerase chain reaction (RT-PCR). To determine which variants are translated in protein we accomplished western blot analysis. Results: The expression of 24-OHase RNA was reduced by more than 50% in RT-PCR as well as in western blotting compared to benign breast tissues. *Discussion:* Breast cancer tissue has a reduced activity of 24-OHase which leads to higher levels of active metabolites of Vitamin D. Alternative splicing of 24-OHase might play a role in regulating levels of the active enzyme. High levels of splice variants might lead to a reduction of the active protein. We found less splice variants in malignant breast cancer tissue. These results correspond with the data found in previous studies we performed in malignant and benign gynaecologic cell lines.

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LIGAND-DIRECTED DELIVERY OF CYTOKINES TO TUMOR VASCULATURE USING NGR/*iso*DGR PEPTIDES

Angelo Corti

Department of Oncology, Cancer Immunotherapy and Gene Therapy Program, San Raffaele Scientific Institute, via Olgettina 58, 20132 Milan, Italy

The use of cytokines, such as tumor necrosis factor-α (TNF) and interferon-γ (IFNγ), in cancer therapy is often limited by strong systemic toxicity and poor efficacy. To improve the therapeutic index of these drugs we have developed, in the last years, a vascular targeting approach based on cytokine fusion with peptides containing Asn-Gly-Arg (NGR) or *iso*Asp-Gly-Arg (*iso*DGR) motives, *i.e.* ligands capable to bind CD13 or specific integrins expressed by angiogenic endothelial cells and, consequently, to deliver these cytokines to tumor neovasculature. Studies in various animal models have shown that the therapeutic index of these fusion proteins is greater than that of non-targeted cytokines. Furthermore, we also observed that systemic administration of very low doses (picograms) of NGR-TNF or *iso*DGR-TNF fusion proteins can increase the penetration of chemotherapeutic drugs in tumors, by altering endothelial barrier function. NGR-TNF is now under investigation in phase II clinical studies for cancer treatment, alone and in combination with chemotherapy. Since early preclinical studies we soon realized that the dose-reponse curve of these peptide-cytokine fusion products is complex, either when used alone or in combination with chemotherapy. For instance, while synergy with chemotherapy can occur with doses of NGR-TNF in the picogram range (about 10^6-fold lower than the LD_{50}) increasing the dose to nanograms leads, paradoxically, to lower responses. Furthermore, coadministration of NGR-TNF with endothelial-monocyte activating polypeptide-II (EMAP-II), an inflammatory cytokine known to sensitize tumor blood vessels to TNF, can induce synergistic pro-apoptotic effects with low-dose EMAP-II (picograms), but not with high doses (nanograms-micrograms). This behavior has been observed also with IFNγ-NGR. Studies on the mechanism of action have shown that high doses of targeted or non-targeted TNF, IFNγ or EMAP-II can activate counter-regulatory mechanisms that efficiently block or counteract cytokine activity. Thus, targeted delivery of low doses of cytokines to tumor blood vessels is a novel strategy for avoiding not only toxic reactions, but also for overcoming counter-regulatory mechanisms.

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A PHASE II MULTICENTRIC PROSPECTIVE TRIAL ON HEPATIC ARTERIAL YTTHRIUM MICROSPHERES AS SALVAGE THERAPY IN UNRESECTABLE, CHEMOREFRACTORY COLORECTAL LIVER METASTASES

Maurizio Cosimelli[1], Pierpaolo Cagol[2], Livio Carpanese[1], Francesco Fiore[3], Daniele Gasparini[2], Onelio Geatti[2], Rita Golfieri[4], Francesco Izzo[3], Rosa Sciuto[1] and Carlo L. Maini[1] on behalf of the Italian Society of Locoregional Therapies in Oncology

[1]Regina Elena National Cancer Institute, Rome; [2]University of Udine; [3]Cancer Institute of Naples; [4]University of Bologna, Italy

Background: Intra-arterial injection of ^{90}Y resin microspheres radiotherapy (SIRT) enables delivery of tumorcidal doses of radiation to malignant tumor, with minimal damage to adjacent tissue. This multicentre phase II study is the first prospective evaluation of SIRT as salvage therapy for patients with colorectal liver metastases who had failed prior oxaliplatin- and irinotecan-based regimens. *Methods:* Eligible patients had a life expectancy of >6 months, adequate hepatic and renal function and an absence of major vascular anomalies and pulmonary shunt >10%. The gastroduodenal and right gastric arteries were embolized before injection of microspheres (median dose 1.7 GBq; range 0.9-2.2) into the hepatic artery by arteriography. *Results:* Patients were enrolled and followed up for a median of 11 months (mos) (range 2-27). Of 50 eligible patients, 38 (76%) had received at least 4 lines of chemotherapy. Most patients had synchronous disease (72%), >4 hepatic metastases (58%) (median size 50 mm; range 8-120), involving 25-50% of the liver tissue (60%) and bilateral spread (70%). Early and late (after 48 h) WHO G1-2 toxic events (mostly fever and pain) were observed in 16% and 22% of patients, respectively. One responding patient died after 60 days due to liver failure. Of 46 patients who were evaluable for response using RECIST criteria, 1 patient (2%) had a complete response (CR), 11 (22%) a partial response (PR), 12 (24%) stable disease (SD) and 22 (44%) progressive disease (PD). In responders (CR + PR + SD), the maximum diameter of nodules diminished to 35 mm. The Kaplan-Meyer overall median survival was 13 (CI 7-18) mos, with a significant difference (p=0.0006) between responders 16 (CI 13-19) mos and PD 8 (CI 4-12) mos. At 2 years, survival was 40.3% and 0% in responders and PD, respectively. The median time to progression (mostly extrahepatic) was 4 (CI 3-5) mos. *Conclusion:* In heavily pretreated patients, ^{90}Y resin microspheres produced an encouraging median survival, with acceptable toxicity, that compares favorably with previous phase II/III studies of chemotherapy regimens used as third- or subsequent lines of therapy.

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ULTRACONSERVATIVE SURGERY AFTER NEOADJUVANT CHEMORADIATION IN LOCALLY ADVANCED RECTAL CANCER

M. Cosimelli, R. Mancini, F. Ambesi Impiombato, C. Garufi, M. Zeuli, G. Paoletti, F. Graziano, I. Sperduti, V. Stigliano and M. Caterino

The Colorectal Disease Management Team, Regina Elena National Cancer Institute, Rome, Italy

Neoadjuvant chemoradiation (CHRT) downstages up to two thirds of low rectal cancer patients (pts.) clinically staged T_{3-4}, any N or any T, node-positive (N+); risk of positive lymph nodes in major responders (pT_0-1) ranges from 0 to 17%. In our experience on 186 consecutive pts who had undergone neoadjuvant chemoradiation for locally advanced, low rectal cancer, the pathologically complete (pT_0) or almost complete (pTmic, pT1) response was 27.9%. Clinical, pathological and biological predicting factors for response such as number of endoscopic quadrants involved, TRG classification, low score of thymidylate aynthase, p53 negativity and bcl-2 positivity were identified. Chances of sphincter-saving surgery and pelvic control of disease were significantly high (88% and 92% respectively) after CHRT (oxaliplatin, capecitabine and 5,040 cGy) and total mesorectal excision surgery (TME); also risk of metastatic mesorectal lymph nodes was particularly low (3.1%) in the 52 pts. pT0-1. A subset of 20 pts. cT0-1 underwent a transanal local excision (LE) for different reasons (elderly or refusal of TME surgery): 11 (55%) were staged pT0, another 8 (40%) pT1 and only one pT2 (5%). No deaths have been observed as yet (median following, 18 months); three local relapses (median time, 9 months) occurred in the pT2 patient and in two other pT1. All the 3 patients are free of disease after a salvage TME sphincter-saving surgery (one node-positive); another patient developed distant metastasis. In conclusion, ultraconservative rectal surgery is feasible and oncologically safe in well-selected rectal cancer pts. after major response to CHRT.

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MECHANISMS OF REDOX-MODULATED RESISTANCE TO APOPTOSIS IN TUMOUR CELLS

Thomas G. Cotter, Christopher Pettigrew, Ruth Naughton and Claire Quiney

Tumour Biology Laboratory, Department of Biochemistry, University College Cork, Ireland

There is increasing evidence within the literature that the decreased susceptibility of tumour cells to stimuli that induce apoptosis can be linked to their inherently increased redox potential. This research work focuses on the PI3-kinase/Akt pathway, and the multiple points along this signalling pathway that may be redox regulated. The PI3-kinase/Akt pathway can influence a cell's sensitivity to death-inducing signals, through direct manipulation of apoptosis regulating molecules or by regulating the activity of key transcription factors. Proteins involved in the control of apoptosis that are directly regulated by the PI3-kinase/Akt pathway include caspase-9, Bad and the transcription factor GSK-3beta. Lately, it is becoming increasingly obvious that phosphatases are a major counter balance to the PI3-kinase/Akt pathway. Phosphatases such as PP2A and PP1alpha can dephosphorylate signalling molecules within the PI3-kinase/Akt pathway, blocking their activity. It is the balance between the kinase activity and the phosphatase activity that determines the presence and strength of the PI3-kinase/Akt signal. This is one reason why any protein modifications that hinder dephosphorylation can increase the tumour survival advantage. One such modification is the oxidation of the sulphydryl group in key cysteine residues present within the active site of the phosphatases. The generation of H_2O_2 by the Nox (NADPH oxidase) system is central to this effect in prostate and CML leukaemia cells.

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REGULATORY MECHANISMS OF VITAMIN D SYNTHESIS AND CATABOLISM FOR COLORECTAL CANCER PREVENTION

Heide S. Cross, Thomas Nittke, Maya Khorchide and Enikö Kallay

Department of Pathophysiology, Medical University Vienna, A-1090, Austria

Occurrence of non-familial sporadic colorectal cancer (CRC) is frequent, especially in rich industrialized countries. High incidence has been related to environmental nutritional factors and progression into clinically manifest disease may take several decades. Epidemiological studies indicate that vitamin D insufficiency may also play a role in its etiology. Vitamin D insufficiency is determined by serum 25-hydroxy vitamin D3 (25-D3) concentration (<75 nmol/l). Lifestyle and nutrition may contribute to insufficiency, though a major part of vitamin D is produced in skin cells by UV-B, and only fatty fish and egg yolk contain vitamin D in appreciable amounts. 25-D3 is converted by hydroxylation in kidney proximal tubule cells to the active metabolite 1,25-dihydroxy vitamin D3 (1,25-D3). Serum 1,25-D3 does not exceed picomole concentrations and seems not to correlate with cancer incidence. Since *in vitro* only nanomolar levels have been shown to display antimitotic activity, we hypothesized that localized 1,25-D3 synthesis could occur in extrarenal tissues, reaching nanomole concentrations, and the prerequisite condition for this is the availability of sufficient 25-D3 serum levels. We demonstrated that indeed there is active 25-D3 1α-hydroxylase (CYP27B1)

in colon mucosal cells and that early during human colon tumor progression expression of the synthesizing enzyme as well as of the vitamin D receptor (VDR) is enhanced. In high-grade undifferentiated tumors this expression is diminished and the vitamin D catabolizing hydroxylase CYP24A1 becomes prominent. Thus enhanced expression of the vitamin D system may be an innate defense against further progression of malignancy, and raising extrarenal local production in organs prone to malignancy could curb progression.

We established that (phyto)estrogens improved expression and activity of CYP27B1, and decreased that of CYP24A1. This could conceivably account for reduced CRC incidence in women compared with men, and also for reduced incidence in soy-consuming countries. Dietary calcium is a well-accepted inhibitor of colonic hyperproliferation. We demonstrated in a mouse model that low dietary calcium led to hyperproliferation and to enhanced expression of CYP24A1 in colon mucosa. Interestingly this occurs only in the right (proximal) colon in both genders. However, only in female mice on low dietary calcium is CYP27B1 as well as VDR expression raised, again only in the right colon. In females, enhanced vitamin D synthesis may override its enhanced degradation since 1,25-D3 measured in colon mucosa was at least doubled. This paralleled raised apoptotic activity.

High expression of the catabolic CYP24A1 in undifferentiated tumors is apparently not associated with gene duplication, but rather with epigenetic regulation. CpG islands in the *CYP24A1* promoter of cells derived from advanced human colon tumors are not methylated whereas those of cells derived from early well-differentiated malignancies are highly methylated and they do not possess *CYP24A1* mRNA or activity. When the latter cells were treated with a demethylation agent, they expressed not only *CYP24A1* mRNA but also the capacity to degrade 1,25-D3.

This demonstrates that colonic 1,25-D3 could be harnessed as a biological weapon against tumor progression by enhancing its mucosal synthesis and by curbing its degradation. While high doses of active 1,25-D3 given to tumor patients generally result in hypercalcemia, improving local production in organs prone to malignancy could slow down progression without leading to enhanced serum levels and to hypercalcemia.

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EFFECT OF AN ALLELIC POLYMORPHISM IN THE DOPAMIN RECEPTOR D2 GENE ON THE RISK OF CERVICAL CANCER

József Cseh[1], István Ember[2], Zsuzsanna Orsós[2], Antal Tibold[2], Zsuzsanna Varga[3], Emese Pázsit[4] and István Kiss[2]

[1]Department of Oncology, Fejér County "Szent György" Hospital, Veszprém;
[2]Institute of Preventive Medicine, Faculty of Medicine, Pécs University of Sciences, Pécs;
[3]Department of Oncotherapy, Pécs University of Sciences, Pécs;
[4]Department of Obstetrics and Gynecology, Diósgyőr Hospital, Miskolc, Hungary

In spite of the decreased mortality rates, cervical cancer is still an important tumor in developed countries, because of its high incidence, including precancerous lesions. Human papillomavirus infection, particularly with high-risk strains is considered to be the most important risk factor for cervical cancer. However, in spite of the infection, the cancer will not develop in several women, which suggests that other risk factors play also an important role in cervical carcinogenesis.

During the recent years a few studies tried to find an association between dopamine metabolism and cancer risks. Since dopamine is an important neurotransmitter, this connection is probably mediated by causing changes in cancer-risk modifying behavioral patterns like alcohol consumption and smoking, but direct molecular effects cannot be excluded, either. Dopamine receptor D2 (DRD2) takes part in the intracellular transmission of dopamine effects. The TaqIA polymorphism (a C32806T substitution) in the DRD2 gene was shown to affect the receptor function, personality traits and the occurrence of certain mental diseases.

In our case-control study we investigated the effect of DRD2 TaqIA polymorphism on the risk of cervical cancer. Altogether 143 women participated in the study. Their HPV status was determined, and their disease progression was also recorded. The HPV positive cases were genotyped for DRD2 TaqIA polymorphism by PCR-RFLP, and allele frequencies were compared between individuals who remained disease-free and who developed cancer or precancerous lesion. Our results indicate a moderate risk modifying effect of the TaqIA polymorphism, which, together with other low penetrance genetic factors, might have an influence on the risk of cervical cancer.

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POLY(ADENOSINE DIPHOSPHATE-RIBOSE) POLYMERASE-1 EXPRESSION IN CUTENEOUS MALIGNANT MELANOMAS AS A NEW MOLECULAR MARKER OF AGGRESSIVE TUMOR

Béla Csete[1], Zsuzsanna Lengyel[1], Zsolt Kádár[1] and Zita Battyáni[1,2]

[1]Department of Dermatology, Venereology and Oncodermatology, University of Pécs, Pécs;
[2]Department of Dermatology, Kaposi Mór Teaching Hospital, Kaposvár, Hungary

Poly(adenosine diphosphate-ribose) polymerases (PARPs) are a family of enzymes which catalyse poly (ADP-ribosyl)ation of DNA-binding proteins and are directly involved in genomic

stability, DNA repair and apoptosis. In this study, we evaluated the immunomorphology of PARP-1 in melanoma and its prognostic importance.

We studied PARP-1 expression by immunohistochemistry in a selected series of 54 primary cutaneous malignant melanoma (CMM). The findings of the present study suggest that the neoplastic progression toward the invasive (both horizontal and vertical) growth phase of CMM cells is characterized by the loss of cleavage of PARP-1, probably signaling an imbalance of the apoptotic process in these cells and leading to further gain to aggression. Overexpression of full-length PARP-1 was correlated with recurrence and progression of the disease and so acts as a promising new biological marker of CMM.

Our study represents the evidence of a direct correlation between the PARP-1–mediated apoptotic process and the biological behavior of CMM.

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LGALS3BP, A TUMOR-ASSOCIATED ANTIGEN, UPREGULATES VEGF IN HUMAN BREAST CANCER: POSSIBLE IMPLICATIONS IN ANGIOGENESIS

Albana Cumashi, Nicola Tinari, Enza Piccolo, Cosmo Rossi, Rossano Lattanzio, Armida D'Incecco, Antonino Grassadonia, Maurizia D'Egidio, Daniela Semeraro, Mauro Piantelli, Clara Natoli and Stefano Iacobelli

Department of Oncology and Neurosciences, University "G. D'Annunzio" Chieti-Pescara, Via Colle Dell'Ara, 6100, Chieti, Italy

Vascular Endothelial Cell Growth Factor (VEGF) represents a key regulator factor on angiogenesis occurring in a variety of malignancies, including breast cancer (1). Similarly, LGALS3BP, a lectin galactoside-binding soluble 3 binding protein, has emerged as a novel feature that may favour breast cancer progression and metastasis, since its high expression levels in patients significantly correlates with poor prognosis (2-3). So far, how LGALS3BP overexpression intervenes in tumor progression and metastasis remains still to be elucidated, but a link between LGALS3BP and tumor angiogenesis has been recently suggested (4). With the aforementioned, we sought to study the possible interplay between LGALS3BP and the proangiogenic molecule, VEGF in breast cancer. To this end, we initially performed immunohistochemical studies on the tissue specimens obtained by biopsies of a group of 40 patients affected by breast carcinomas, indicating that LGALS3BP expression in tumor tissues was directly correlated with VEGF expression in 72.5% of cases. Moreover, *in vitro* experiments showed that LGALS3BP treatment could increase VEGF mRNA levels in MDA-MB-231 breast cancer cells, but not in non tumorigenic HBL-100 cells. This evidence suggested that LGALS3BP might represent a new upregulator factor for VEGF expression/activity in human breast cancer. For a further confirmation, a stable down-regulation of LGALS3BP in MDA-MB-231 cells was obtained by using siRNA expression plasmids: the protein level found in the Conditioned Medium (CM) of LGALS3BP-silenced cells was 80% less when compared to the CM-derived from oligo control cells. Very interestingly, Real Time PCR studies demonstrated that the level of VEGF mRNA expression in LGALS3BP-silenced MDA-MB-231 was significantly lower in respect to that expressed by oligo control cells (55%, p=0.011). Similar results were obtained also by measuring VEGF protein level as indicated by using either confocal microscopy or immunoprecipitation studies. Because VEGF is a principal regulator factor for endothelial cells, we also studied the exogenous LGALS3BP-induced effects on human umbilical vein endothelial cells (HUVECs). Our results show that LGALS3BP specifically upregulated VEGF mRNA expression, but did not affect the expression level of other important growth factors for HUVEC. In addition, LGALS3BP, but not the denaturated protein, could activate the VEGF-promoter transfected in endothelial cells. Finally, supernatants collected from LGALS3BP-silenced or oligo control MDA-MB-231 cells, were tested for their capability to induce *in vitro* HUVEC tubulogenesis. Our data indicated an evident decrease of the number of tubuli found onto Matrigel when HUVEC were incubated with the supernatant of cells that lack LGALS3BP, thus signifying a promoting role of this protein on angiogenesis *in vitro*. Taken together, these results lead to the conclusion that LGALS3BP might be depicted as a novel candidate that promotes angiogenesis. Further studies are in progress for investigating any potential effect of antibodies directed against human LGALS3BP on the *in vivo* growth of MDA-MB-231 breast cancer cell xenografts and on the related tumor-angiogenesis. This information could lead to the development of novel antiangiogenic therapy.

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HUMAN PAPILLOMAVIRUS (HPV) INFECTION ACCOUNTS FOR AN INCREASE IN THE INCIDENCE AND THE BETTER PROGNOSIS IN TONSILLAR CANCER IN SWEDEN

Tina Dalianis

Department of Oncology-Pathology, Karolinska Institutet, Cancer Center Karolinska, Karolinska University Hospital R8:01, S-171 76 Stockholm, Sweden

We previously reported a parallel three-fold increase in the incidence of tonsillar cancer and in the proportion of human papilloma virus (HPV)-positive tumours in the Stockholm area, between 1970-2002. Notably, while only 23% of the cases were HPV positive in the 1970s, 68% were HPV-positive during 2000-2002, when testing 203 available pre-treatment tonsillar cancer biopsies with both general and type-specific HPV PCRs and sequencing. Moreover, 87% of the HPV-positive cases were HPV type 16. These results suggested that HPV infection, with a dominance of HPV 16, was responsible for the increase in the incidence of tonsillar cancer, a disease also more common in men than women in Sweden.

In continuation, we analysed the clinical outcome in these 203 patients and reported that overall disease-free survival was longer in patients with HPV-positive tumours as compared to patients with HPV-negative tonsillar cancer. However, a correlation between prognosis and high viral load, previously reported in a pilot study, could not be confirmed. Nonetheless, 94% of all tested HPV 16-positive tumours expressed E7 mRNA and the majority of these also expressed E6 mRNA, indeed supporting the oncogenic role of HPV in tonsillar cancer.

In parallel, we conducted a smaller study in collaboration with colleagues from the Metaxas Cancer Hospital in Piraeus, Greece, where we analysed an additional 103 pre-treatment samples from 115 patients with head neck cancer, and of these 28 were diagnosed with tonsillar cancer between 1992-2007. Again, we see a tendency for an increase in the proportion of HPV-positive tumours from 17% during the years 1992-1998 as compared to 50% between 2000-2007, although unfortunately the numbers of tumours were too few to allow for a statistical significance.

Since these studies, the incidence in tonsillar cancer seems to have increased further in Sweden and particularly in the Stockholm area. The aim of the present study was therefore to examine the proportion of HPV-positive tonsillar cancer cases between 2003-2007 within the Stockholm area. During this period, 150 pre-treatment tonsillar cancer biopsies were collected and the samples were analyzed in a similar way to the samples of the 203 patients above. In this study, we found that the proportion of HPV-positive tumours between 2003-2007 was around 80%, with the proportion of HPV-positive tumours being 86% during 2006-2007.

In summary, the data support that the continued increase in incidence of tonsillar cancer seems to be due to a continued increase in the proportion of HPV-positive tumours, at least in Sweden. Based on recent data, that patients with HPV-positive tonsillar cancer do better than patients with HPV-negative tonsillar cancer, we suggest that the presence or absence of HPV in tonsillar cancer should be considered when tailoring treatment. Finally, the more recent, very strong association of HPV with tonsillar cancer should also possibly pave the way for the development of preventative strategies, such as possible vaccination against HPV infection in both young women and men.

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REACTIVE OXYGEN SPECIES (ROS) COMPRISE A MOLECULAR TARGET IN PREVENTION OF ORAL CELL CANCER

Steven D'Ambrosio, Chunhua Han and Haiming Ding

Department of Radiology, Division of Radiobiology, The Ohio State University, Columbus, Ohio, USA

Redox is a normal physiological process balancing the intracellular levels of endogenously and exogenously produced oxidants and antioxidants. The levels of reactive oxygen species (ROS) are often maintained in a narrow range and perturbing the balance between pro- and antioxidants in cells can lead to apoptosis. Due to high levels of metabolism and other factors, cancer cells often exhibit high levels of ROS. This may contribute to their high proliferation rates, genomic instability and promote invasion by killing adjacent normal cells. To survive, cancer cells maintain a delicate balance between pro- and antioxidants and perturbing this balance may offer an opportunity for cancer prevention. A number of studies indicate that many cancer preventative and therapeutic agents induce apoptosis *via* ROS. It is thought that ROS act as signals to initiate apoptosis *via* either the intrinsic and/or extrinsic pathways.

We have been investigating the cancer protective affects of phytochemicals extracted from a number of fruits. In the present study, we used organic extracts prepared from avocados to determine ROS mechanisms involved in prevention using a human oral cancer cell culture model. Phytochemicals extracted from avocado flesh using chloroform (D003 extract)

selectively inhibit the growth and induce apoptosis in premalignant and malignant, but not normal, human oral epithelial cell lines. A number of cellular and molecular approaches were used to determine mechanisms responsible for the selective activity of avocado extracts. The premalignant and malignant oral cell lines contained significantly higher basal levels of ROS than did the normal oral cell lines. Upon treatment of the cancer cell lines with the D003 extract, ROS levels increased 3-fold and induced apoptosis. ROS levels only increased 1.3-fold in the apoptosis-resistant normal oral cell line. The increased levels of ROS induced by D003 in the cancer cell lines appeared to be mediated *via* mitochondrial complex I in the electron transport chain. The involvement of ROS in the selective killing of the cancer cell lines was further substantiated when these cell lines became resistant to D003-induced apoptosis upon reduction of cellular ROS levels by *N*-acetyl-L-cysteine (NAC). NAC also delayed the induction of apoptosis in dominant negative FADD-expressing cancer cell lines, suggesting the role of ROS in this signaling pathway. To further confirm the role of ROS in extract induced apoptosis, we transformed the resistant normal oral epithelial cell line with HPV16 E6 or E7. These cell lines exhibited characteristics of the oral cancer cell lines, including increased levels of ROS, and sensitivity to apoptosis induced by the D003 extract.

In summary, the data suggest that: i) ROS may be regulatory molecules activated by the phytochemicals in avocado; and ii) perturbing the ROS levels in human oral and other cancer cells may be a key factor in selective apoptosis and molecular targeting for chemoprevention by phytochemicals.

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DEVELOPMENT OF APTAMERS AS TARGETED RADIOPHARMACEUTICALS FOR THE DIAGNOSTIC IMAGING AND RADIOTHERAPY OF BREAST CANCER

C. Da Pieve[1], A. Perkins[2] and S. Missailidis[1]

[1]Chemistry Department, The Open University, Walton Hall, Milton Keynes, MK7 6AA;
[2]Department of Medical Physics, Medical School, University of Nottingham, Nottingham, NG7 2RD, UK

Aptamers have shown great potential as novel targeted radiopharmaceutical entities for the diagnosis and medical imaging of disease. They offer reduced immunogenicity, good tumour penetration, rapid uptake and clearance compared to their monoclonal antibody counterparts. In previous work, we have reported on the labelling of such aptamers against breast cancer related biomarkers with radionuclide ligands.

We have successfully conjugated selected aptamers against the protein core or the tumour glycosylated MUC1 glycoprotein to MAG2 and labelled them with ^{99m}Tc, for the diagnostic imaging of breast cancer. The conjugation is achieved using standard peptide coupling reactions between an amino modification on the aptamer and the carboxylic group on the ligands. An efficient and convenient labelling of the aptamer with short half-life radioisotopes was achieved as the last step of the synthesis. Both conjugation and labelling reactions were monitored by HPLC. The labelled aptamers were separated from free ^{99m}Tc using ultrafiltration, before injection and imaging for analysis of their tumour localising potential and pharmacokinetic properties. For the analysis of the pharmacokinetic properties of the aptamer-radionucleotide conjugate, we have used gamma-camera imaging in MCF-7 breast cancer tumour model systems.

Stability tests showed that the aptamer-chelator conjugates have strong ^{99m}Tc binding properties and the resulting complexes are highly stable *in vivo*, both in terms of nuclease degradation and leaching of the metal. We analysed the uptake of two different radiolabelled aptamers, selected against the naked MUC1 tandem repeat sequence and the tumour glycosylated Tn antigen respectively, in the tumour at 3, 5, 16, and 24 hours after the injection. It has been previously shown that conjugation of aptamers to high molecular weight polyethylene glycol (PEG) modifies the pharmacokinetic properties of the radiolabeled product, allowing the complex longer circulation times and thus offering improved tumour imaging properties. This approach gave us further possibilities for development of efficient targeted radiopharmaceuticals for breast cancer imaging and therapy.

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ENVIRONMENTAL OESTROGENS AND BREAST CANCER

Philippa D. Darbre

School of Biological Sciences, University of Reading, Reading RG6 6AJ, UK

The established role of oestrogen in the development, progression and treatment of breast cancer raises questions concerning a potential contribution from the many chemicals in the environment which enter the human breast and which possess oestrogenic activity. This may include the plant-derived phytoestrogens, synthetic pharmacological oestrogens of oral contraceptives/hormone replacement therapy, and man-made oestrogen-mimicking chemicals. A range of ubiquitous environmental contaminants (organochlorine pesticides and polychlorinated biphenyls) and compounds widely used in the domestic environment (alkyl phenols, phthalates, polybrominated diphenyl ethers) possess oestrogenic activity and have been measured in human breast adipose tissue and/or in human milk. However, an extensive array of cosmetic

chemicals are applied to the human breast area on a daily basis and are left on the skin allowing for accumulation in the underarm and upper outer breast area. Our work is showing that an increasing number of these cosmetic chemicals possess oestrogenic activity and are measurable in human breast including parabens, aluminium salts, triclosan, phthalates, sunscreens, polycyclic musks and other fragrance compounds. The disproportionate number of breast cancers in the upper outer quadrant of the breast, just the local area to which these chemicals are applied, remains strong supportive evidence of a causal link (Darbre, Anticancer Res *25*: 2543, 2005). This lecture will review evidence for a functional role of the combined actions of environmental oestrogens in the rising incidence of breast cancer.

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A MULTI-TARGETED THERAPY FOR CANCER: AN "AGE-OLD" REMEDY FOR AN "AGE-OLD" DISEASE

Tanya Das[1], Gaurisankar Sa[1], Charles Tannembaum[2] and Samit Chattopadhyay[3]

[1]Division of Molecular Medicine, Bose Institute, Kolkata, India;
[2]Department of Immunology, Cleveland Clinic, Ohio, USA;
[3]National Centre for Cell Science, Pune, India

Cancer is a multifactorial disease that requires modulation of multiple pathways and multiple targets. In the recent past, dietary plant polyphenols, *e.g.*, theaflavins and curcumin, have provided opportunities to develop strategies for curing cancer by directly or indirectly altering specific cellular targets. We attempted to develop a multiple signal modulation therapy of cancer by targeting signalling pathways leading to (i) apoptosis and (ii) metastasis. Our approaches centred around tumour suppressor protein p53. Using human cancer cells of varying p53 status, we found a definitive co-relation between p53 status and the signalling pathways targeted by these polyphenols. Apoptosis was induced in wild-type p53-expressing cancer cells by p53-mediated Bax trans-activation and stimulation of intrinsic death pathway, while in cancer cells containing mutant p53, activation of death receptor-dependent extrinsic apoptotic pathway and inhibition of survival pathway initiated intrinsic mitochondrial death cascade. In HPV-infected cancer cells, these polyphenols resisted E6-dependent p53 degradation not only by down-regulating E6 *via* activation of the transcription repressor Cux/CDP, but also by up-regulating MAR-binding protein SMAR1. SMAR1 in turn inhibited Mdm2-mediated p53-degradation and stabilized p53 through phosphorylation at its Ser15 residue with simultaneous deacetylation of this tumour suppressor. Moreover, these plant polyphenols were found to retard the migration of wild-type p53-expressing cancer cells more efficiently than those with mutant p53. A search for the underlying mechanism revealed disruption of the membrane lipid raft-associated integrin-signalling pathway. The outcome of this study might expand our knowledge in developing a "new" strategy of multi-targeted therapy of the "age-old" disease cancer utilizing the "age-old" remedies.

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DNA REPAIR ENZYME POLYMORPHISM AND RISK OF OVARIAN CANCER IN ISFAHAN

Mehdi Nikbahkt Dastjerdi

Department of Anatomical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Aims: The *ERCC2* gene encodes a DNA repair enzyme that has multiple regulatory cellular functions. The *ERCC2* polymorphism Lys751Gln may alter the capacity for DNA repair, which could affect the risk of certain types of cancer. *Methods:* We examined whether the Lys751Gln polymorphism was associated with the risk of ovarian cancer in Isfahanian women by analysing the genotype frequencies in 86 patients with ovarian cancer and 120 cancer-free controls. *Results:* The Gln /Gln genotype was associated with a 53% increased risk of ovarian cancer. *Conclusion:* Our results demonstrated that *ERCC2* polymorphisms might be potential risk markers for ovarian cancer in Isfahan.

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PTEN AND BCL-2 EXPRESSION IN COLORECTAL CARCINOMAS WITH MICROSATELLITE INSTABILITY

Mehdi Nikbahkt Dastjerdi

Department of Anatomical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Aim: The aim of this study was to examine the relationship of PTEN (phosphatase and tensin homolog deleted on chromosome 10) and bcl-2 protein expression to microsatellite instability, as well as to other clinicopathological variables in colorectal adenocarcinomas. *Materials and Methods:* We evaluated the expression patterns of antiapoptotic Bcl-2 and PTEN proteins, as potential prognostic markers in colorectal cancer by immunohistochemical staining. To identify high-frequency microsatellite instability (MSI+) specimens, we performed single-strand conformation polymorphism-based analysis for BAT26. A total of 100 colorectal specimens were evaluated. *Results:* Increased expression of bcl-2 (>50% cells) was seen in 17 specimens (17%) and loss of PTEN was seen in 14 specimens (14%). No significant correlation was observed between the proteins or with clinicopathological factors. Loss of PTEN was more frequent in MSI-positive tumors (8/24

[32%]) than in negative tumors (6/76 [8%]; p=0.012). *Conclusion:* Our data demonstrate that bcl-2 overexpression occured in a subset of colorectal carcinomas, while loss of PTEN often was associated with the MSI phenotype.

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_TP53_ CODON 72 POLYMORPHISM AND MICROSATELLITE INSTABILITY IN SPORADIC COLORECTAL CANCER

Mehdi Nikbahkt Dastjerdi

Department of Anatomical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Aim: The polymorphic variants at codon 72 of the *p53* gene, encoding either proline or arginine at residue 72, produces marked change in the structure of p53. From the evidence that the DNA mismatch repair system and p53 interact to maintain genomic integrity, we hypothesized that the codon 72 variation may influence the prevalence of microsatellite instability, a feature of malignancies associated with mismatch repair deficiency in sporadic colorectal cancer. *Materials and Methods:* We investigated the frequency of microsatellite instability in three genotypes of *P53* codon 72 using genomic DNAs from 190 paraffin blocks of the sporadic colorectal adenocarcinomas by testing the BAT-26 marker. *Results:* MSI analysis revealed that 27.6% of the tumors were MSI-positive and 72.4% showed no change (MSI-negative). The frequency of microsatellite instability in the arginine/arginine, arginine/proline and proline/proline genotypes were 17.9%, 66.1% and 16% respectively. A significant difference in distribution of MSI was found for the arginine/proline genotype as compared with (grouped) arginine/arginine and proline/proline genotypes (p=0.05). *Conclusion:* Our findings suggested that colorectal adenocarcinomas arising in individuals with *p53* codon 72 heterozygosity (arginine/proline) are preferentially prone to microsatellite instability more than other genotypes.

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ANNEXIN A1 EXPRESSION IN COLORECTAL CANCER

Mehdi Nikbahkt Dastjerdi

Department of Anatomical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Aim: The role of annexin A1 (ANXA1) in tumor development and progression is controversial. We investigated ANXA1 expression and determined its clinical significance in colorectal cancer. *Methods:* Blocks containing primary colorectal cancer, lymph node metastases, and adjacent normal mucosa specimens were obtained from 120 Isfahanian patients. Expression of ANXA1 in these specimens was analyzed using immunohistochemistry. *Results:* Complete loss of ANXA1 expression was observed in 63% of the 120 primary tumors and 85% of the nodal metastases. Loss of ANXA1 expression was significantly associated lymph node metastasis and poor histological differentiation. *Conclusion:* ANXA1 expression decreased significantly as colorectal cancer progressed and metastasized, suggesting the importance of ANXA1 as a negative biomarker for colorectal cancer development and progression.

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DETECTION OF LYMPH NODE MICROMETASTASES IN EARLY BREAST CANCER

Mehdi Nikbahkt Dastjerdi

Department of Anatomical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Aim: The purpose of this study was to examine the usefulness of the biopsy of the lymph nodes for effective detection of lymph node micrometastasis in early breast cancer and to clarify the spread of lymph node micrometastasis. *Methods:* One hundred local and regional lymph nodes from 30 patients with early breast cancer were evaluated by staining with haematoxylin and eosin and immunohistochemically for antibodies to pancytokeratin (AE1/AE3) and cytokeratin 14. *Results:* The immunohistochemical tests detected occult micrometastases in 16% of the lymph nodes that were negative by haematoxylin and eosin staining. *Conclusion:* Routine systematic lymphadenectomy with immunohistochemical detection of lymph node micrometastasis contributes to identification of a larger population at risk of early breast cancer.

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A NEW NEGATIVE BIOMARKER FOR BREAST CANCER DEVELOPMENT

Mehdi Nikbahkt Dastjerdi

Department of Anatomical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Aim: The role of annexin A1 (ANXA1) in tumor development and progression is controversial. We investigated ANXA1 expression and determined its clinical significance in breast cancer. *Methods:* Blocks containing primary breast cancer, lymph node metastases, and adjacent normal mucosa specimens were obtained from 100 Isfahanian patients. Expression of ANXA1 in these specimens was analyzed using immunohistochemistry. *Results:* Complete loss of ANXA1 expression was observed in 59% of the 100 primary tumors and 75% of the nodal metastases. Loss of ANXA1 expression was significantly associated lymph node metastasis and poor

histological differentiation. *Conclusion:* ANXA1 expression decreased significantly as breast cancer progressed and metastasized, suggesting the importance of ANXA1 as a negative biomarker for breast cancer development and progression.

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DO SHARK LIVER OILS INFLUENCE THE GROWTH OF NORMAL AND TRANSFORMED MAMMALIAN CELLS IN CULTURE?

B.C. Davidson[1], G. Cliff[2], W. Prinz[1] and D. Rottanburg[1]

[1]School of Physiology, University of the Witwatersrand Medical School, Johannesburg, Gauteng;
[2]Natal Sharks Board, Umhlanga Rocks, KwaZuluNatal, South Africa

Several reports have indicated an extremely low incidence of cancer in sharks, although other reports indicate carcinogenesis in some shark species. Many authors have published reports of polyunsaturated fatty acids exerting growth inhibitory effects on transformed cells but not normal cells in culture. It has thus been hypothesised that n3 polyunsaturated fatty acids, present in high concentrations in many marine oils, especially shark liver oils, may exert anticarcinogenic effects, and these n3 polyunsaturated fatty acids have been shown to have anticarcinogenic effects on mammalian carcinomas. The aim of this study was to assess whether shark liver oil, from four Indian Ocean shark species, exerted antiproliferative effects on certain transformed and normal mammalian cells in culture, and to assess whether the ratio of n3 to n6 polyunsaturates influenced the results. Although certain concentrations of the oils did induce growth inhibition, this was not consistent between the oils nor did they show a concentration dependence with either transformed or normal cells. Moreover, the ratio of n3 to n6 did not seem to be a significant factor. Fatty acid mixtures mimicking the composition of the shark liver oils also did not induce any significant profiles of growth inhibition. It would seem unlikely for the liver oils from these four species of shark to be of use in anticancer therapy.

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***RUNX1* TRANSLOCATIONS IN MALIGNANT HEMOPATHIES**

Etienne De Braekeleer, Claude Férec and Marc De Braekeleer

Faculté de Médecine et des Sciences de la Santé & INSERM U-613, 22 avenue Camille Desmoulins, CS 93837, F-29238 Brest cedex 3, France

The *RUNX* gene family includes three evolutionarily conserved genes (*RUNX1, RUNX2* and *RUNX3*) encoding transcription factors involved in cell lineage differentiation during development and various forms of cancer. The *RUNX1* gene, located in chromosome 21q22, is crucial for the establishment of definite hematopoiesis and the generation of hematopoietic stem cells in the embryo. It contains a "Runt homology domain" (RHD) and a transactivation domain. RUNX1 can act as activator or repressor of target gene expression depending upon the large number of transcription factors, coactivators and corepressors that interact with it.

Three modes of leukemogenesis due to acquired alterations of the *RUNX1* gene have been recognized: point mutations, amplification and translocations. Some translocations have been shown to be recurrent, whereas others have been only reported in a few cases or in a sole case.

At present, 32 partner chromosomes have been described but the partner gene has solely been identified in 17 translocations at the molecular level. Most of the translocations involving *RUNX1* lead to the formation of a fusion transcript made of the 5' region of *RUNX1*, including the RHD, fused to the 3' region of a partner gene, with the exception of *RUNX1-ETV6* in which the 3' sequences of *RUNX1*, including the RHD, is fused to the 5' region of *ETV6*, including its promotor. Three *RUNX1* translocations (retaining RHD) that are fused out of frame to partner genes are also known. All the translocations that retain RHD but remove the transcription activation domain have a leukemogenic effect by acting as dominant negative inhibitors of wildtype *RUNX1b* in transcription activation.

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DELTANp63 EXPRESSION IS ACTIVATED BY BETA-CATENIN

Charlotte Ruptier[1], Alexia De Gaspéris[1], Aurélie Granjon[1], Ilta Lafosse[1], Philippe Tanière[2], Hong Shi[2], Audrey Petitjean[2], Estelle Taranchon[2], Catherine Cavard[3], Violaine Tribollet[1], Thibault Voeltzel[1], Stéphane Ansieau[1], Alain Puisieux[1], Pierre Hainaut[2] and Claude Caron de Fromentel[1]

[1]INSERM U590, Université Lyon, Centre Léon Bérard, Lyon;
[2]Molecular Carcinogenesis and Biomarkers Group, International Agency for Research on Cancer, Lyon;
[3]INSERM U-567 CNRS UMR 8104, Institut Cochin, Paris, France

TP63 gene is, with *TP53* and *TP73*, a member of the *TP53* family. It may encode both long (TAp63) and truncated (DeltaNp63) isoforms, by the use of promoters P1 and P2, respectively. TAp63 isoforms act as transcription factors. They activate genes involved in cell cycle arrest or apoptosis. DeltaNp63 isoforms lack the main transactivation domain but retain the DNA-binding domain. Thereby, they are able to bind

p53 responsive elements (p53RE) and to prevent both p53 and TA isoforms from binding on p53RE. This latter property could be a mechanism of tumour initiation and/or progression in some tumour types.

When deregulated in tumours, as squamous cell carcinomas (SCC), *TP63* expression mainly results in the accumulation of DeltaNp63 variant. The amplification of *TP63* gene, observed in about 30% of SCC, cannot explain all the cases of accumulation observed. Therefore, we hypothesized that DeltaNp63 overexpression could also result from P2 promoter deregulation.

Several sites were already identified in the P2 promoter, among them a p53RE, suggesting a regulation of P2 promoter by p53. Actually, it has been demonstrated that p53 represses P2 promoter independently from the p53RE, but through CAAT boxes present near the TATA box.

By in silico analysis, we identified two sites for TCF/LEF transcription factors in the P2 promoter. Therefore, we tested the modulation of P2 by beta-catenin (the co-activator of TCF/LEF), but also by p53 and DeltaNp63 itself. We confirmed the repression of P2 by p53 and its activation by DeltaNp63alpha, both independently of the p53RE. Moreover, we showed an activation of P2 by beta-catenin. All these effects are through a direct binding of p53, DeltaNp63 and beta-catenin on P2 promoter. We are currently mapping the TCF/LEF binding sites involved in the activation of DeltaNp63 by beta- catenin.

Since stabilization and delocalization of beta-catenin is frequently observed in tumours, we searched for the association between DeltaNp63 overexpression and beta-catenin delocalization in oesophageal SCC. Beta-catenin delocalization was found in 13 out of the 16 samples tested (81%). Furthermore, 11 of these 13 tumours also exhibited DeltaNp63 accumulation (84%). Our results suggest that beta-catenin could be responsible for DeltaNp63 overexpression in SCC. The functional cross-talk between these two proteins is currently under investigation, in order to determine if they are able to promote together abnormal cell proliferation and tumorigenesis.

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NARRATIONS OF "INQUIRING AND CONFUSED" CANCER PATIENTS

Sandra Degli Esposti Elisi

Researcher and Professor of Cultural Anthropology, Department of Sciences of Education "Giovanni Maria Bertin", Alma Mater Studiorum, University of Bologna, Italy

Contemporary anthropological and sociological literature on health places attention frequently on patients' everyday life narrations; through these, they make sense of their pathology, their recovery, to the necessity to share their own experiences, their own convictions and expectations.

In particular in our modern time often these narrations are mediated *via* new technologies, I would point out how these become patient's genuine diary of life in which he tells about the daily challenge of cancer and the suffering, but also of the search for information, advice and aid. Narrations that are important to outline the figure of a "inquiring" but at the same time "confused" patient in the face of the multiplicity of inputs he receives.

In this context research has investigated some telematic 'spaces' (born at the border of magazines on health and wellness), from discussion forums to chat-lines, from sites dedicated to different pathologies to blogs to outline the patient's universe, that the health professionals and of weaving of consequent relations.

Member of: AISEA, Italian Association of Ethnoanthropological Sciences; SSE/SGE, Societé Suisse d'Ethnologie; PARACELSUS, Centre of Social Studies on Health, Care and Quality of Life, University of Ferrara, Italy.

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SINGLET OXYGEN ($^1O_2$) FORMED BY RESVERATROL AS A CAUSATIVE FACTOR OF CYTOTOXICITY

Despina Fotiou, Stelios Fotiou and George Deliconstantinos

Department of Experimental Physiology, University of Athens Medical School, Athens 11527, Greece

Resveratrol (3,4,5-trihydroxystilbene), a natural phytoalexin found in the grape skin and wine, expresses a wide range of pharmacological properties including antioxidant activity. Our present experiments showed that resveratrol as a stable radical gives a concentration dependent luminol chemiluminescence response (~8×10^8 cpm) while the antioxidant Trolox (~1 mM) suppresses and shifts the chemiluminescence spectrum to the right at ~4×10^5 cpm, clearly showing its scavenging activity. In chemiluminescence studies, in the presence of a stable amount of nitrosoglutathione (~10^{-6} M), low concentrations of resveratrol (10^{-6} M) act as prooxidants releasing singlet oxygen ($^1O_2$) that reacts with nitric oxide (NO) originating from nitrosoglutathione producing peroxynitrite ($ONOO^-$). Beta-carotene acts as an acceptor of $^1O_2$ formed by resveratrol as tested in fluorometric studies (a) by inhibition of hydroxylation of terepthalic acid and (b) by an increase of the oxidation rate of resveratrol shown by the decrease of its fluorescence spectrum Ex 330, Em 374).

Synaptosomes isolated from rabbit brain release nitric oxide (NO) and resveratrol increases NO synthase (NOS) activity while NO was converted to $ONOO^-$ verifying the formation of singlet oxygen ($NO + {}^1O_2 \rightarrow ONOO^-$). Similar results were also obtained with xanthine oxidase (XO) using pterin as a substrate and/or oxypurinol as an inhibitor (XO). Fluorescence polarization studies using diphenyl-hexatriene (TMA-DPH)

showed a decrease in the fluorescence polarization of TMA-DPH incorporated in the synaptosomal membranes from r=0.205 to r=0.157, consistent with an increase in membrane fluidity thus relieving the physical constrain imposed by the lipids on enzymes, pumps, channels *etc*. The oxidative damage to biomolecules induced by $^1O_2$ and the protective effects of beta-carotene were evaluated by the enhanced peak chemiluminescence response of resveratrol in the presence of DNA and/or beta carotene. The concentration-dependent enhancement of resveratrol-DNA complex peak chemiluminescence observed was decreased in the presence of beta-carotene clearly showing the damaging effects of resveratrol due to the formation of $^1O_2$.

In conclusion we showed that at low concentrations, resveratrol acts as a prooxidant while at high concentrations as an antioxidant and that damages of biomolecules by resveratrol could result from the formation of singlet oxygen that may act alone and/or in combination with nitric oxide to form the cytotoxic $ONOO^-$.

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OXIDATIVE STRESS CAUSED BY ULTRAVIOLET C IN RAT SKIN MICROVESSELS AND MICROSOMES WITH RESPECT TO SKIN PHOTOAGING AND CANCER

Despina Fotiou, Stelios Fotiou and George Deliconstantinos

Department of Experimental Physiology, University of Athens Medical School, Athens 11527, Greece

The aim of the present study was to investigate whether UVC irradiation of the skin could result in the formation of oxygen and/or nitrogen free radicals. When heme-iron is present in the skin it can act both as a strong oxidative agent itself and as a source of continuous free radical production having therefore the potential to cause skin damages.

In our experiments UVC irradiated microvessels isolated from rat skin produce NO and $ONOO^-$. When irradiation takes place $ONOO^-$ is attached to OH group in the Fe^{3+} of hematin to form the stable radical hematin-Fe-$[(OH)ONOO]^{2-}$ as follows:

Hematin-Fe-NO + O_2^- → Hematin-Fe-$(OH)ONOO^{2-}$

The stable radical hematin-Fe-$(OH)ONOO^{2-}$ was detected by UV diode array spectroscopy at 420 nm. $ONOO^-$ was determined either spectrophotometrically at 302 nm or fluorometrically by dihydrorhodamine 123 oxidation to rhodamine at excitation 500 nm and emission at 528 nm.

Microsomes isolated from rat skin released nitric oxide (NO), which was enhanced by arginine and reduced by nitro-arginine. UVC irradiation activated both NO synthase and xanthine oxidase activities leading to the formation of $ONOO^-$. Fluorescence polarization studies using diphenyl-hexatriene (TMA-DPH) for the estimation of the membrane fluidity, showed an increase in the fluorescence polarization of TMA-DPH incorporated in the microsomal membranes from r=0.179 to r=0.600 consistent with a decrease in membrane lipid fluidity suggesting a constrain imposed by the lipid bilayer on the function of enzymes, pumps and/or channels.

As shown herein UVC exposure causes the generation of the free radical Fe-$[(OH)ONOO-]^{2-}$ and NO and oxygen free radicals in the skin microcirculation and skin layers. The vast majority of people daily spent some time in the daylight and because it is not the conventional "sun bathing", it would not have been expected that daily, suberythemal exposure to the sun causes photodamage and possibly cancer as a result, in part, of UV-mediated increase of skin oxidative stress. Therefore, our studies are broadly viewed as refocusing the concept of preventing photoaging and skin cancer using a topical application of a composition having a Fenton reaction blocker.

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CANCER CELLS PHAGOCYTOSIS BY MAST CELLS

Filippo della Rovere[1], Angelo Granata[1], Maurizio Monaco[1] and Giacomo Basile[2]

[1]U.O.C. di Chirurgia Toracica, [2]Anatomia Patologica, Università degli Studi di Messina, Azienda Ospedaliera Universitaria "G. Martino", Messina, Italy

The data in the literature confirm the hypothesis that incidence of cancer disease is lower in allergic individuals. In a previous study, we too confirmed that histamine plays a role in protecting against cancer. Briefly, Sprague-Dawley rats were first inoculated with histamine *i.p.*, followed by 1,000 cells of Yoshida ascites sarcoma. In 80% of the rats treated in this way, no cancer disease onset was reported. In a different study, histamine and selenium were assayed in subjects with lung cancer. In tumor subjects, significant decreases in both histamine and selenium were reported ($p<0.0001$) as compared with healthy control subjects.

Our present study focused on mast cell activity in breast carcinoma. We found that mast cell numbers were higher in high hormone receptive cancer, and we focused mainly on cytolysis of neoplastic cells that help the body to protect itself from oncogenic aggression. It was demonstrated that cancer cells are first surrounded by the mast cell pseudopodium; they are then engulfed in the mast cell cytoplasm, where toxic granulations trigger the cytolytic process. The phagocytosed cell progressively loses its chromatic and volumetric characteristics until complete achromia and almost complete reduction of its volume and consistency occur. The cell nucleus soon degenerates to pyknosis, and is no more detectable. The phagocytosis can occur simultaneously in different neoplastic cells.

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TUMOUR DORMANCY IN BREAST CANCER

Romano Demicheli

Istituto Nazionale Tumori, Milan, Italy

The review analyzes the recent evolution of two paradigms related to the development of breast cancer metastases. The continuous growth model is required to yield to an interrupted growth model, the tantamount of which are episodes of "tumour dormancy". Primary tumour removal, usually considered as intrinsically beneficial, proves to be able to perturb metastatic homeostasis and to result, for some patients, in the acceleration of metastatic cancer spread. Paradigm evolution is supported by a growing body of findings from experimental models and is required to explain breast cancer recurrence dynamics for patients undergoing surgery without or with adjuvant chemotherapy.

Classical models that were proposed to explain breast cancer metastasis dynamics after primary tumour removal assumed the implicit hypothesis that tumours must always grow. The concept of uninterrupted growth failed to explain findings from both local and local plus distant recurrences. In particular, the bimodal recurrence pattern, which presents an early peak at the second year after surgery, a second peak at about 5 years and a tapered plateau-like tail extending up to 15 years, should be explained. This pattern is independent of the seeded organ, and may be observed in all metastatic sites. On the contrary, a new dormancy-based model of metastasis development was found to better fit clinical data. According to this model, metastatic tumour may either continuously grow or even sojourn in two dormant states, *i.e.* single cells and avascular micrometastases, with orderly transitions between these two dormant states eventually resulting in progressive appearance of clinical metastases. Moreover, some precipitating event at the time of primary tumour surgical removal may have a triggering effect. In spite of a century of investigations, the effects of primary tumour surgical removal on metastases have practically been ignored by clinicians. Single cells may be induced to proliferate *via* the conversion of non-cycling G0 cells or by switching avascular micro-metastatic foci to active angiogenesis. These processes occur to different extent in pre- and post-menopausal patients. The model found confirmation by the analysis of the recurrence risk for patients receiving adjuvant CMF, where the recurrence reduction occurred at specific, temporally separate recurrence clusters at the first and third year, for both menopausal statuses.

The proposed dormancy-based metastasis development model implies some kind of control on tumour growth from the microenvironment, some kind of homeostatic effect upon distant metastases. These concepts are poorly understandable within the classical frame, where cancer is a genome-driven disease, *i.e.* a cell-autonomous irreversible process and where the tumour microenvironment is an idle bystander, sometimes forced to provide factors supporting tumour progression. However, all these views (epitheliocentric somatic mutation paradigm, irreversibility of the neoplastic phenotype, insignificant role of the microenvironment) have been challenged by experimental evidence both *in vitro* and *in vivo*. A new image of breast cancer is emerging. Cells that we label "cancer cells" go on with their peculiar ability to have cross-talk with the environment. This trait accounts for the neoplastic behaviour, ranging from quiescence to open growth, in different sites and/or at different times. Tumour dormancy and the counterintuitive consequence of primary tumour removal have a logical place in this context. The traditional image of breast cancer is changing.

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PROTEOMIC ANALYSIS OF TELOMERASE INHIBITION BY TELOMERE SPECIFIC LIGANDS

Gabriel Mazzucchelli[1], Valérie Gabelica[1], Nicolas Smargiasso[1], Frédéric Rosu[1], Marie-Claire De Pauw-Gillet[2], Jean-François Riou[3] and Edwin De Pauw[1]

[1]Laboratory of Mass Spectrometry and [2]Histology and Cytology Laboratory; CART, GIGA, University of Liège, BAT. B6C, allée de la Chimie, 3, 4000 Liège 1, Belgium; [3]Regulation et Dynamique des Genomes, Museum National d'Histoire Naturelle, USM 503, INSERM U565, CNRS UMR 5153, Paris, France

Telomeres consist of protein complexes and repeated 'TTAGGG' double strand DNA sequences ended by a 3' single strand DNA of the same sequence. Progressive telomere shortening is observed *in vitro* upon cell divisions and with ageing *in vivo*. At a critical telomere length, shortened telomeres trigger a permanent growth arrest known as replicative senescence. Telomerase is an RNA-dependent DNA polymerase that extends telomeres by adding 'TTAGGG' repeats. It consists of a functional RNA component (hTR) which serves as template and a catalytic protein (hTERT) with reverse transcriptase activity. The expression of hTERT alone is sufficient for the immortalisation of cells. Telomerase is highly expressed in tumor cells but at very low level in most somatic cells. These observations make the telomerase an attractive target for anticancer strategies. One of these strategies relies on the use of drug candidates able to stabilize the particular telomere G-quadruplex DNA structures. The stabilization of these structures makes the telomere inaccessible for telomerase and thus inhibits telomerase activity.

The effect of the hTERT transfection was first studied on the proteome of human WI38 fibroblast cells (1). Then, the proteome alteration response of hTERT transfected WI38 cells

induced by the treatment of two G-quadruplexes ligands, telomestatin and TMPyP4, was analyzed. Both compounds can inhibit telomerase but have different selectivity for the different G-quadruplexes structures.

Proteome analysis of the treated cells reveals that TMPyP4 induces much more protein expression alterations than telomestatin probably due to its poor selectivity. TMPyP4 induces especially a drastic down expression of the hnRNPs, a modulation of the proteasome pathway, an apparent decrease of the translation and an over expression of several molecular chaperones. Telomestatin induces in particular an over expression of the protein BCL2A1 which is involved in drug-resistance of cancer cells and a probable increase of the translation. Both treatments have a common effect particularly on the molecular chaperone CCT (down expression), HSP90 alpha (over expression) and hnRNP D (down expression). The protein HSP90 alpha is also over expressed in hTERT transfected cells compared to parental cells. This protein is already a promising anticancer target protein due to its central role in oncogenesis and in telomerase activity regulation.

1 Mazzucchelli *et al*: Proteome Science *6*: 12, 2008.

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LNCaP PROSTATE CANCER IMAGING WITH BIOLOGICALLY FUNCTIONALIZED GOLD NANOPARTICLES IN 2D AND 3D CELL CULTURES

D. Schol[1], M. Fléron[1], J.F. Greisch[1], M.C. De Pauw-Gillet[1], E. De Pauw[1], M. Jaeger[2], M. Frenz[2], S.A. Eccles[3], J. Bamber[3], S. Frosini[4], L. Masotti[4], M. Fournelle[5] and R. Lemor[5]

[1]University of Liège, Liège, Belgium;
[2]University of Bern, Bern, Switzerland;
[3]The Institute of Cancer Research, Sutton, Surrey, England;
[4]El.En. S.p.A., Calenzano, Italy;
[5]Fraunhofer-Institut fuer Biomedizinische Technik IBMT, St. Ingbert, Germany

A major challenge in oncology is to develop more accurate imaging assessments. The ADONIS Project intends to prove the concept of using optoacoustic imaging with biologically functionalized nanoparticles as an integrated biosensor based imaging system for the production of specific and sensitive data for accurate diagnosis of prostate cancer. This concept involves using contrast agents which upon photoactivation induce the local heating of their environment, generating pressure waves that are detectable by piezoelectric transducers.

One of the main objectives of this project is to produce and validate a versatile lab system composed of functionalized nanoparticles for diagnosis of different superficial and accessible cancers, *e.g.* prostate cancer. Gold nanorods have been synthesized and functionalized with antibodies targeting specific antigens on cancer cell lines. The foremost challenge consists in synthesizing rod-like nanoparticles absorbing about 1064 nm, the spectral range where biological tissues absorb the least. A wet chemical approach in solution, using surfactants as dynamic template and silver nitrate as growth inhibitor, is used for synthesis. Once the particles have been synthesized, the surfactant is replaced by a biocompatible polymer for use in *in vitro* tests. The polymer-coated nanoparticles are then coupled with an antibody directed against the cancer cells to guarantee the selective detection of the particles.

Prostate Specific Membrane Antigen (PSMA), a transmembrane protein considered as a suitable biomarker for prostate cancer, was selected as the primary target. Recognition and successful binding of the biosensor to PSMA is demonstrated by various techniques using cell monolayers and 3D cell cultures. PSMA localization on the LNCaP cell membranes was identified by immunocytochemistry (HRP, Q-Dots). Backscattered electron (BSE) microscopy and two-photon luminescence imaging proved that the biosensor is bound to the viable and fixed cells expressing PSMA.

Gold particles attached to cancer cells serve as contrast agents for optoacoustic detection. The concept of detecting PSMA-expressing tumours using this integrated optoacoustic biosensor system was confirmed on LNCaP spheroids (cell aggregates) in gelatine phantoms. This system is currently being tested on *in vivo* human tumour xenograft animal models and in the future will be tested on human tumour biopsies.

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CLINICAL AND BIOLOGICAL ASPECTS OF PERITONEAL MESOTHELIOMAS

Marcello Deraco[1], Nadia Zaffaroni[3], Federica Perrone[2], Dario Baratti[1], Raffaella Villa[3], Shigeki Kusamura[1], Genny Jocollé[2], Antonello D. Cabras[2] and Silvana Pilotti[2]

[1]Department of Surgery, Fondazione IRCCS Istituto Nazionale Tumori, Milano;
[2]Department of Pathology, Fondazione IRCCS Istituto Nazionale Tumori, Milano;
[3]Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale Tumori, Milano, Italy

Background: Diffuse malignant peritoneal mesothelioma (DMPM) is a rare and rapidly lethal neoplasm. In recent years, the combination of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (CRS+HIPEC) has resulted in a significant survival improvement, as compared to historical controls. Little is known about DMPM genetic and molecular features. In the present study, we assessed new prognostic indicators and therapeutic targets in a large series of DMPM undergoing CRS+HIPEC. *Methods:* From a prospective database of 86 cases, we selected 66 DMPM. Cases with well-

differentiated histology or second malignancies were excluded. All the patients underwent peritonectomy procedures and closed-abdomen HIPEC with cisplatin and doxorubicin. The prognostic significance of age, sex, carcinomatosis extension, completeness of cytoreduction (CC) and HIPEC drug schedule was tested by multivariate analysis. We evaluated the expression of members of the Inhibitor of Apoptosis Protein (IAP) family (survivin, IAP-1, IAP-2, X-IAP) and the presence of telomere maintenance mechanisms (telomerase activity and alternative lengthening of telomeres [ALT]). In 15 patients, EGFR, PDGFRA and PDGFRB expression and phosphorylation were immunohistochemically and biochemically analysed and automatically sequenced. The cognate ligand expression was investigated by real-time PCR. Additionally, we explored RTK downstream pathways status through mutational and biochemical analysis of *PI3KCA* gene PTEN/AKT, ERK, mTOR and its effector S6. *Results:* Median follow-up, overall (OS) and progression-free survival (PFS) were 30.5 (range: 1-118), 40 and 17 months. Median. CC independently correlated to OS and age to PFS. IAPs were simultaneously up-regulated in a high percentage of DMPMs (survivin was present in >90% of cases). Survivin gene knockdown in mesothelioma cells resulted in a significant and time-dependent decline of *in vitro* growth and enhanced rate of spontaneous and drug-induced apoptosis. At least one telomere maintenance mechanism was present in 86% of DMPMs. Telomerase activity correlated to poor OS and PFS, whereas ALT failed to significantly affect clinical outcome. Immunohistochemical and western blot analyses showed EGFR, PDGFRA and PDGFRB expression and activation in most cases. Autocrine loop activation of these receptors was suggested in all cases by the expression of the related cognate ligands, in absence of receptor gain of function mutations. No PI3KCA mutations were found, while all DMPMs showed expression of PTEN and expression/activation of AKT, ERK, mTOR and S6. *Conclusion:* CRS+HIPEC is associated to encouraging survival results. Both telomere maintenance mechanisms, telomerase activity and ALT are present in DMPM and differentially affect prognosis. EGFR, PDGFRA and PDGFRB are promising molecular targets for tailored treatments.

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PERITONEAL SURFACE MALIGNANCIES: FROM BIOLOGY TO INNOVATIVE TREATMENTS

Marcello Deraco, Dario Baratti, Barbara Laterza, Domenico Sabia and Shigeki Kusamura

Peritoneal Malignancies Unit, Department of Surgery, Fondazione IRCCS Istituto Nazionale Tumori, Milano, Italy

Peritoneal surface malignancies (PSM) represent the end-stage of many intrabdominal tumors. Better knowledge of biology, natural history and pattern of dissemination (PSM remain confined within the peritoneal cavity for most of their clinical course), makes a locoregional approach combining Cytoreductive Surgery (CRS) and Hyperthermic Intra-peritoneal Chemotherapy (HIPEC) attractive. The intraperitoneal chemotherapy maximizes the dose intensity and simultaneously minimizes systemic toxicity. Hyperthermia increases tumor cell chemosensitivity. Due to the limited HIPEC penetration into tumor tissue, peritonectomy procedures and multi-visceral resections are required for cytoreduction down to microscopic/minimal residual disease.

Colorectal cancer is the second most common cause of cancer death, with peritoneal carcinomatosis (PC) occurring in 50% of patients. Median survival in PC patients is about 6 months. A prospective randomized study has shown the superiority of CRS+HIPEC over standard treatment.

Ovarian cancer accounts for the greatest number of deaths from gynecological malignancy. 75% of cases are diagnosed at stage III/IVwith a median survival of 36 months.

According to several phase II studies the treatment of ovarian cancer PC with CRS+IPHP has provided 5-year survival rates of 15-63%.

Peritoneal mesothelioma is an uncommon tumor with a median survival of less than 1 year. CRS and HIPEC have reportedly resulted into a survival improvement to 32-94 months. Pseudomyxoma peritonei is a rare condition originating from an appendiceal mucinous tumor. This minimally invasive disease is associated to poor long term prognosis, due to its tendency of locoregional relapse. CRS and HIPEC have been advocated as the treatment of choice.

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BRAIN TUMOURS *EX VIVO* AND THEIR CLINICAL APPLICATION

Leo De Ridder

Department of Histology, Ghent University, Belgium

Introduction: Several special features are specific for brain tumours: poor definition followed by infiltrative growth,rich in various cell types,progression towards malignancy, nearly complete absence of metastasis and finally brain tumour kill at the site of origin. These characteristics can be analysed *ex vivo* especially infiltration and progression. Although morphological as well as other observations on biopsies give a framework for research, investigations outside the body are not only useful but mandatory. *Materials and Methods:* Several model systems are available. Animal and human brain tumours can be propagated over varying periods in cell, tissue and organotypical cultures. However since the development of culture techniques, it is clear that the closer the model resembles the *in vivo* situation the more reliable the results are With this in mind, it is essential to select a given *ex vivo*

method to answer a given question. *Results:* Monolayers of primary cultures and cell lines, spheroidal aggregates, organotypical and confronting cultures will answer different questions concerning the biological behaviour of brain tumour derived cells. Monolayer cultures of cell lines answer general metabolic problems, primary monolayer cultures will give answers for the individual tumour cells and pharmacological substances can be tested but are not applicable for infiltration studies. Tumour cell aggregates, formed by reassociation of tumour derived cells, consist of viable cells including non transformed normal brain cells. They are tridimensional and spheroidal. These aggregates can be used for testing antimitotic and radioprotective substances at the individual level and every single tumour can be evaluated for its proliferation characteristics. Organotypical cultures are tumour fragments freshly collected from the surgical amphitheatre. They are difficult to keep *in vitro* as they are contaminated by necrotic material. On the other hand, confrontations between tumour derived aggregates and host tissue aggregates seem to answer the problems of infiltration and proliferation. Different variations in confrontation cultures are available. Heterologous and autologous confrontations are at hand. The most promising technique might be the confrontation of brain tumour aggregates with aggregates of normal cells of the patient the tumour is derived from (called: homologous confrontation). The optimal condition is not yet realised. *Conclusion:* The actual *ex vivo* techniques allow to evaluate the effects of therapy at the individual level, to distinguish the radioprotective capacity of substances, to analyse the infiltrative and proliferative capacity of the individual brain tumour and to study basic mechanisms of infiltration.

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DYNAMIC REGULATION OF LOW MOLECULAR WEIGHT PROTEIN TYROSINE PHOSPHATASE (LMW-PTP) TRANSIENT NATURE OF ACTIVITY ENSURE THE GM-CSF DEPENDENT STAT5 ACTIVATION

Roberta R.R. de Sousa, Karla C. S. Queiroz, Gweeny M. Fuhler, Carmen V. Ferreira and Maikel P. Peppelenbosch

Department of Cell Biology, University of Groningen Antonius Deusinglaan, Groningen, the Netherlands

LMW-PTP (Low Molecular Protein Tyrosine Phosphatase) is frequently overexpressed in various human cancers. LMW-PTP localizes predominantly to the cytoplasm and functions as phosphatase, but its function in cellular signaling remains unknown. In this work we investigated the role of LMWPTP in JAK/STAT pathway in GMCSF dependent erythroleukemic cells. LMWPTP activity is temporally dynamic modulated by GMSCF. TF1 cells deprived of GM-CSF for 2 h and stimulated with GM-CSF for 5 min show a 5-fold decrease in LMWPTP activity, coinciding with binding of the inactive phosphatase to STAT5. This initial phase is followed by a 2-fold up-regulation of LMWPTP activity after 20 min of GM-CSF stimulation, and a release of STAT5 binding. Interestingly, we demonstrate for the first time that LMW-PTP is present in the nucleus in an inactive state, and shuttles to the cytosol upon GM-CSF stimulation. These results suggest a dual role for LMW-PTP, in which an inactive form of the protein binds to STAT5 in the cytosol followed by activation of the phosphatase enzymatic activity and STAT5 dephosphorylation. Hence LMW-PTP seems a cardinal mediator of GM-CSF-dependent STAT5 activation, ensuring the transient nature of the response.

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CISPLATIN OTOTOXICITY – FROM DOSE TO MOLECULAR BIOLOGY

Dirk Deuster

Department of Phoniatrics and Pediatric Audiology, University Hospital of Muenster, Kardinal-von-Galen-Ring 10, D-48149 Muenster, Germany

Cisplatin is effectively used for the treatment of several childhood malignancies, but its use is limited by its ototoxicity. It includes a usually permanent sensory hearing loss, tinnitus, and alterations in vestibular function. The risk estimates for ototoxicity after cisplatin range from 23 to 53% depending on the criteria used and the diligence of the search. To answer specific questions, such as individual tolerance, a hearing loss classification relating to intensity and the affected frequencies are required. We developed our own classification system based on pure tone audiometry, which detects hearing loss earlier and maps progression of hearing loss more precisely than the existing high frequency classifications. This classification system is now used in all our research.

In animals, damage of various structures of the inner ear like hair cells, stria vascularis, and spiral ganglion could be observed. The mechanisms of cisplatin-induced ototoxicity are poorly understood so far. High rates of cisplatin delivery, cumulative dosage, pre-existing sensorineural hearing loss, age <5 were identified as risk factors for ototoxicity, but apart from these factors interindividual susceptibility to the ototoxicity was observed in clinical studies and animal tests. So genetic predispositions could be possible causes for ototoxocity: i) Oxidative stress has been implicated in cisplatin ototoxicity. Therefore, differences in protection mechanisms against oxidative stress may be one possible reason for the individual susceptibility. We found significant inter-group difference of glutathione S-transferase, an enzyme, which plays an important role in protecting cells from the deleterious effects of oxidative stress. ii) Moreover, genetic variants in transporters for platinum uptake may be responsible for the individual platin tolerance. We found differences in a non-

synonymous single nucleotide polymorphism (SNP) at the megalin gene as another possible risk factor for ototoxicity. At present, we search specific transporters and their inhibition.

Prevention of cisplatin-induced hearing loss needs both more knowledge about the mechanisms of ototoxicity and early detection with professional audiological examinations. We will decide in favour of an interdisciplinary team to investigate further aspects of cisplatin-induces ototoxicity.

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HIGH LEVEL AMPLIFICATION AND OVEREXPRESSION OF FOXA1 AND NKX2-1 IN PULMONARY CARCINOMAS CORRELATE WITH UNFAVOURABLE PROGNOSIS

Lena S. Deutsch

Klinik und Poliklinik für Allgemein- Viszeral- und Thoraxchirurgie, Universitätsklinikum Hamburg Eppendorf Martinistrasse 52, 20246 Hamburg, Germany

Background: Lung cancer is the leading cause of cancer death worldwide. The failure of clinical staging in prediction of the course of disease in many patients shows the need to consider additional predictive factors. Several chromosomal regions are amplified or deleted in lung tumors, but little is known about the underlying genes, which could be important mediators in tumor formation or progression. *Patients and Methods:* High-level amplifications were screened by array-CGH and verified by fluorescence *in situ* hybridization (FISH) and the protein expression of two amplified genes, *FOXA1* and *NKX2-1*, was studied on a TMA containing 613 tumor samples. *Results:* 40% of the lung adenocarcinomas showed narrow high level amplifications. A narrow high-level amplicon at 14q13.3-q21.1 containing *FOXA1* and *NKX2-1*, two important genes for lung morphogenesis, was found in two cases by array-CGH. FISH analysis showed 19%/18% high-level amplifications of *FOXA1* and *NKX2-1* respectively, and 27%/35% of all tumors were positive for NKX2-1 and FOXA1 protein expression. Patients with distant metastases showed significantly higher expression of FOXA1 than patients without distant spread (p=0.003). Time to metastasis was significantly shorter in patients with high level expression of NKX2-1 than in patients with low level or no expression of NKX2-1 (p=0.032), especially in LCLC (p=0.008). *Conclusion:* The results of our study of a large series of lung tumors indicate a negative prognostic function of FOXA1 and NKX2-1 in lung cancer, especially in LCLC. NKX2-1 could be a useful marker for selection of patients who should undergo systemic treatment for prevention of early systemic spread.

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HUMAN ANTIBODY AND KUNITZ DOMAIN-BASED PROTEASE INHIBITORS FOR CANCER THERAPY

Laetitia Devy[1], Art Ley[1], Robert C. Ladner[1], Shafaat A. Rabbani[2], Clive R. Wood[1] and Daniel T. Dransfield[1]

[1]Dyax Corp., 300 Technology Square, Cambridge, MA, USA;
[2]Department of Medicine, McGill University Health Centre, Montreal, QC, Canada

We have used phage display libraries of both fully human antibodies and variants of a Kunitz domain derived from human tissue factor pathway inhibitor (TFPI) to select potent and selective inhibitors of proteases. These have allowed us to assess the contributions of a number of proteases to cancer progression in preclinical models. The success of this approach and the therapeutic potential of these protease inhibitors will be illustrated by our work with plasmin and matrix metalloproteinase-14.

Plasmin is a serine protease predominantly present in the body in its inactive zymogen form (plasminogen). Its activation mainly occurs locally, at the tumor site by urokinase overproduced by cancer or stromal cells. Using phage display, we have identified DX-1000, a variant Kunitz domain derived from TFPI which is a specific and high affinity inhibitor of plasmin (Ki 99 pM). We increased the molecular mass of DX-1000 by chemically coupling four 5 kDa polyethylene glycol (PEG) moieties in order to improve its pharmacokinetic properties. 4PEG-DX-1000 efficiently blocks plasmin-mediated proMMP-9 activation on cells while not significantly affecting haemostasis and coagulation *in vitro*. In a human breast cancer MDA-MB-231 xenograft model, 4PEG-DX-1000 treatment resulted in a significant reduction of primary tumor growth and a decreased incidence of metastases. Together, our results demonstrate the potential of plasmin inhibitors for blocking cancer growth and metastases.

MMP-14 is a membrane-bound zinc endopeptidase that has been proposed to play a central role in tumor growth, invasion and neovascularization. Besides cleaving matrix proteins, MMP-14 activates proMMP-2 leading to an amplification of pericellular proteolytic activity. To examine the contribution of MMP-14 to tumor growth and angiogenesis, we used DX-2400, a highly selective MMP-14 inhibitory antibody. DX-2400 blocked proMMP-2 processing on tumor and endothelial cells, inhibited angiogenesis and slowed tumor progression and formation of metastatic lesions. This combination of potency, selectivity and *in vivo* activity demonstrate the potential of a selective MMP-14 inhibitor for the treatment of solid tumors.

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STROMAL MYOFIBROBLASTS DRIVE INVASIVE CANCER GROWTH

Olivier De Wever

Laboratory of Experimental Cancer Research, Department of Radiotherapy and Nuclear Medicine, Ghent University Hospital, 9000 Gent, Belgium

Tumor-associated myofibroblasts drive tissue invasion, a hallmark of malignancy (4). The origin of myofibroblasts at the tumor invasion front remains controversial although fibroblasts and bone marrow-derived precursors are considered to be the main progenitor cells. To better understand the mechanisms underlying such effects, we established a heterotypic model of human colon tumor-derived myofibroblasts in co-culture with human colon cancer cells (HCT8/E11), using three-dimensional collagen type-I and Matrigel matrices. We analysed the myofibroblast secreted proteins using a combination of proteomics and antibody arrays. We identified two convergent proinvasive agents secreted by myofibroblasts: namely scatter factor/hepatocyte growth factor (SF/HGF) and the TGF-β-upregulated extracellular matrix glycoprotein tenascin-C (TNC), each of which is necessary though not sufficient for invasion. Myofibroblast-stimulated invasion into collagen type I is characterized by a change from a round, nonmigratory morphotype with high RhoA and low Rac activity to an elongated, migratory morphotype with low RhoA and high Rac activity (2). The myofibroblasts are themselves invasive and this activity is stimulated by TGF-β. N-cadherin is implicated in the invasion response of myofibroblasts (3). In conclusion, the mutual interaction between cancer cells and myofibroblasts is dependent on multiple invasive growth promoting factors (through direct cell-cell contacts and paracrine signals) (1). Our data predict that inhibitors directed at this reciprocal molecular and cellular crosstalk will have therapeutic applications for targeting the invasive growth of human primary tumors and their metastatic spread (5).

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INDUCTION OF P21^{WAF1} EXPRESSION AND WILD-TYPE P53 BY *GYNURA PROCUMBENS* LEAVES IN ORAL CARCINOGENESIS (*IN VIVO* STUDY)

Dewi Agustina

Oral Medicine Department, Faculty of Dentistry, Gadjah Mada University,
Jalan Denta, Sekip Utara, Jogjakarta 55281 Indonesia

Anticarcinogenic effect of *Gynura procumbens* leaves has been evidenced in various types of cells either related with its antiproliferative effect or apoptotic induction. Proliferative inhibition is one of mechanisms to inhibit malignancy. The *p53* gene can activate the transcription of downstream effector genes such as *p21^{WAF1}* to induce cell cycle arrest. The aim of this study was to elucidate the anticarcinogenic potency of *G. procumbens* leaves through the induction of *p21^{WAF1}* and *p53* expressions in chemical carcinogen induced-oral carcinogenesis. Ninety-two male Sprague Dawley rats were divided into 11 groups consisting of control groups and groups treated with the chemical carcinogen, 4 nitroquinoline 1-oxide, and by ethanolic extract of *G. procumbens* leaves. Chemical carcinogen was administered thrice weekly for 8, 16 or 24 weeks, whereas the extract was given twice weekly in certain periods commencing either before or after the carcinogen administration. At the end of the 36th week, histopathological examination (H&E) and immunohistochemical analysis using labeled streptavidin biotin method to detect the expression of

$P21^{WAF1}$ and P53 were conducted. Histopathological analysis showed that the ethanolic extract of *G. procumbens* leaves had an oral anticarcinogenic effect in initiation phase either through its preventative (Group II) or prophylactic effect (Group III). Inhibitory mechanism of the ethanolic extract of *G. procumbens* leaves in oral carcinogenesis occurred by inducing $P21^{WAF1}$ expression. This mechanism was demonstrated by the expression of $P21^{WAF1}$ in tissues which P53 expression was not found. From these results, it is apparent that the ethanolic extract of *G. procumbens* leaves could inhibit oral carcinogenenesis through its preventive and prophylactic effects in initiation phase by inducing $P21^{WAF1}$ expression independently of wt P53.

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P90 RIBOSOMAL S6 KINASES: POTENTIAL NEW TARGETS FOR PROMISING ANTICANCER THERAPIES?

C. Dimas

Laboratory of Pharmacology-Pharmacotechnology, Basic Sciences Center, Biomedical Research Foundation of Academy of Athens (BRFAA), Athens, Greece

The p90 ribosomal S6 kinase (RSK) is a family of serine/threonine kinases of the MAPK (mitogen-activated protein kinase) pathway. The family is constituted of four genes in humans and other mammals (RSK1, 2, 3 and 4). Those enzymes are composed of two distinct and functional kinase domains, located in the carboxy-terminal (CTKD) and another one located at the amino-terminal domain (NTKD) that are activated by a series of sequential phosphorylation events. RSKs function as mediators of ERK signal transduction and have been found so far to be expressed in all cell lines and tissues studied. Numerous substrates have been reported such as GSK3β (proliferation and metabolism), p27kip1 (CDK-cyclin inhibitor), bad (pro-apoptotic protein), IκB and p65 (NFκB-pathway). Thus, studies so far suggest that RSKs may promote cell survival by inactivating apoptotic effectors and mediating cell growth and proliferation by regulating factors involved in transcription and mRNA translation.

Among RSK isoforms, rsk2 mutations and dysfunction have been linked in humans to Coffin-Lowry Syndrome (CLS, X-linked mental retardation syndrome) that is often accompanied by dysmorphisms in face and digits and by progressive skeletal deformations. Recent studies point towards a link between RSK activity and cancer as they demonstrate that RSK1 and 2 are overexpressed among different types of cancer. The recent identification of specific RSK inhibitors, such as small molecule inhibitors and RNAi, may help to shed light on the role of these enzymes in oncogenesis and their potential usefulness as targets for the development of novel anticancer drugs.

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VISUALIZATION OF ANGIOGENESIS IN BRAIN TUMORS USING ^{123}I-VASCULAR ENDOTHELIAL GROWTH FACTOR SCINTIGRAPHY

Elke Dimou[1], Georg Widhalm[2], Alexander Bertalanffy[2], Karl Roessler[2], Christian Nasel[3], Peter Angelberger[4], Christine Marosi[5], Robert Dudczak[1] and Shuren Li[1]

[1]Department of Nuclear Medicine, Medical University of Vienna;
[2]Department of Neuro-Surgery, Medical University of Vienna;
[3]Department of Radiology, Medical University of Vienna;
[4]Austrian Research Center, Seibersdorf;
[5]Department of Internal Medicine I, Division of Oncology, Medical University of Vienna, Vienna, Austria

Aim: Vascular endothelial growth factor (VEGF) is a major angiogenic factor. VEGF receptors have been shown to be over-expressed in a variety of tumor vessels including glioblastoma, which may provide the molecular basis for a successful use of radiolabeled VEGF as tumor angiogenesis tracer. In this study we investigated the use of ^{123}I-VEGF as angiogenesis tracer for imaging brain tumors *in vivo*. *Methods and Results:* SPECT examinations were performed 10 minutes after intravenous administration of ^{123}I-VEGF (191±15 MBq) and 18 hours post injection in 20 patients with brain tumor. Glioblastomas were visualized in 7 of 8 patients (88%) shortly after application of ^{123}I-VEGF and were still clearly shown 18 hours post injection. Negative scan results were obtained in one patient with a small glioblastoma size (diameter <2.0 cm) and in 3 patients with benign glioma as well as in 5 patients with glioblastoma after receiving radiotherapy and /or chemotherapy. Weak positive results were obtained in 3 patients with brain lymphoma or other tumors. No side-effects were observed in patients after *i.v.* administration of ^{123}I-VEGF. *Conclusion:* Our results indicate that ^{123}I-VEGF scintigraphy may be useful to visualize the angiogenesis of brain tumors and to monitor the treatment response.

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GENETICS IN RENAL CANCER

Martina Olivero, Annalisa Lorenzato and Maria Flavia Di Renzo

Laboratory of Cancer Genetics, Department of Oncological Sciences at the Institute for Cancer Research and Treatment (IRCC), University of Torino Medical School, SP 142, Km. 95, 10060 Candiolo, TO, Italy

Cancer is a genetic disease, due to the accumulation of mutations in genes (oncogenes and tumour suppressor genes) that control the balance between cell birth and cell death. In some cases these are germline and inherited, while the large majority are somatic mutations. Interestingly, in most cases, such as in inherited kidney cancer syndromes, the same genes cause both inherited and sporadic (non-inherited) forms of cancer.

As the molecular basis of disease continues to be elucidated, familial cancer syndromes, which consist of a range of neoplastic and non-neoplastic features, are emerging. The usual pathway of referral to a genetics clinic or familial cancer centre is *via* either a surgeon or an oncologist, when high-risk features that suggest a possible hereditary basis for the presenting cancer are recognized. Traditionally, these high-risk features include more than two family members with similar types of cancer over two or more generations, a young age of onset, and more than one synchronous or metachronous tumour. These features are effective in ascertaining a substantial proportion of families with hereditary cancer. However, there are a range of familial cancer syndromes that are not easily detected and can remain undiagnosed when history and examination are not extended to include cancer in other sites and non-malignant features.

Identification of predisposition to develop cancer is particularly important in the case of inherited kidney cancer syndromes, as it provides individuals and their families with the opportunity to undertake early surveillance for malignant complications that might in time be shown to undertake nephron-sparing surgery and to improve outcomes. Kidney cancer with diverse aggressiveness has been associated with germline mutations of one of the following genes: *VHL, MET, FH, BHD* or *TSC*. The genetic mechanism, histopathology and clinical history of kidney cancer associated with each of the above gene mutation will be discussed.

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NOVEL ROLES OF ETS1 IN CANCER PROGRESSION

Jürgen Dittmer

Clinic for Gynaecology, University of Halle, Halle (Saale), Germany

Ets transcription factors, such as Ets1, play an important role in cancer progression. Ras-responsive Ets1 promotes invasion of tumor cells by orchestrating the expression of ECM-degradating proteases. Ets1 is also involved in tumor-induced neo-angiogenesis. A search for additional targets for Ets1 revealed a link between Ets1 and Rho-GTPases. Ets1 up-regulates the expression of Rho-GDIβ, an inhibitor of Rho-GTPases. In primary breast cancer, Ets1 and Rho-GDIβ are coexpressed. An analysis of Rho-GDIβ function in breast cancer cells revealed that Rho-GDIβ has a dual effect. By inhibiting cellular migration, Rho-GDIβ acts tumor-suppressively; by inducing the expression of the oncogene Cox-2 Rho-GDIβ likely promotes tumor progression. Rho-GDIβ regulates *Cox-2,* by cooperating with the Rho-GTPase guanine exchange factor Vav1, which is coexpressed with Rho-GDIβ in primary breast cancer. Despite its positive effect on Cox-2, Rho-GDIβ was not found to negatively affect the survival of breast cancer patients, rather, patients with Rho-GDIβ-positive breast cancer tend to have a better outcome. This tendency was more pronounced when breast tumors were negative for Rho-GDIα, a ubiquitously expressed close relative of Rho-GDIβ. In contrast to Rho-GDIβ, Rho-GDIα significantly increased survival of breast cancer patients. This effect, however, was absent when Rho-GDIβ was expressed. It seems that both the Ets1-regulated Rho-GDIβ and Rho-GDIα are capable of slowing down cancer progression. However, the effect of Rho-GDIβ is compromised by its ability to induce Cox-2 expression.

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TUMOR RECOVERY BY ANGIOGENIC SWITCH FROM SPROUTING TO INTUSSUSCEPTIVE ANGIOGENESIS AFTER TREATMENT WITH PTK787/ZK222584 OR IONIZING RADIATION

Ruslan Hlushchuk[1,5], Oliver Riesterer[2], Oliver Baum[1], Jeanette Wood[3], Guenther Gruber[4], Martin Pruschy[2] and Valentin Djonov[1,5]

[1]Institute of Anatomy, University of Bern, Bern;
[2]Department of Radiation Oncology, University Hospital, Zurich;
[3]Novartis Pharma AG, Basel;
[4]Department of Radiation Oncology, University Hospital, Bern;
[5]Institute of Anatomy, University of Fribourg, Fribourg, Switzerland

Background: Inhibitors of angiogenesis and radiation induce compensatory changes in the tumor vasculature not only during treatment, but also after its cessation. *Methods:* To assess the response to the treatment, the tumors were analyzed immediately after cessation of therapy and during the recovery phase. Mammary carcinoma allografts were investigated by vascular casting, electron, light, confocal microscopy, and immunoblotting after fractionated irradiation or treatment with the VEGF-receptor tyrosine kinase inhibitor, PTK787/ZK222854. *Results:* Irradiation and antiangiogenic therapy had similar effects on the tumor vasculature in the recovery phase. Both treatments reduced the tumor vascularization, particularly in the tumor medulla. After cessation of therapy, the tumor vasculature expanded predominantly by intussusception, with a plexus composed

of enlarged sinusoidal-like vessels containing multiple transluminal tissue pillars. Tumor revascularization originated from preserved SMA-positive vessels in tumor cortex. Quantification revealed that recovery was characterized by an angiogenic switch from sprouting to intussusception. The up-regulated SMA-expression during the recovery reflected the recruitment of SMA-positive cells for intussusception as a part of the angioadaptive mechanism. Tumor recovery was associated with a dramatic decrease (by 30-40%) in the intratumoral microvascular density, probably as result of intussusceptive pruning, surprisingly with only a minimal reduction of the total microvascular (exchange) area. Therefore, the vascular supply to the tumor was not severely compromised as demonstrated by HIF-1-alpha expression. *Conclusion:* Irradiation and antiangiogenic therapy causes a switch from sprouting to intussusceptive angiogenesis as part of a compensatory response to preserve and restore perfusion. Intussusceptive angiogenesis, with an associated low endothelial proliferation rate and permeability, may represent an escape mechanism and account for the development of resistance to therapy, as well as the rapid recovery of tumor vasculature after cessation of therapy.

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CHEMOSENSITIVITY TESTING: PREDICTING THE UNPREDICTABLE

Ralph Dollner

Department Otorhinolaryngology, Head and Neck Surgery, Rikshospitalet University Clinic Oslo, N-0027 Oslo, Norway

Objective: According to a recent assessment of the working group for technology assessment by the American Society of Clinical Oncology (ASCO), a reliable assay for detecting chemosensitivity in tumour tissue and selecting chemotherapeutic agents for individual patients is still missing (Schrag *et al*. 2004, JCO). Therefore, it seems advisable to reassess the major issues for a reliable test system. Particularly for testing solid tumours, three basic considerations should be addressed: (i) how to provide "reproducibility" in performing chemosensibility tests with unique biopsy specimens, (ii) whether inevitable non-malignant cells present in processed biopsies affect the analysis of the malignant cells, and (iii) whether single biopsy analyses allow us to draw conclusions valid for a presumably cellular very heterogeneous tumour mass. *Materials and Methods:* The introduction of flavin-protecting cell culture methods was a breakthrough, since for the first time it was possible to perform chemosensitivity tests that are not affected by photochemical "chaotropic" artefacts (Granzow *et al*. 1995, Cancer Res). Subsequently an *ex vivo* test has been developed to address the above mentioned issues (Dollner *et al*. 2004, Anticancer Res). This new assay was performed on 60 tumour biopsy specimens of head and neck squamous cell carcinomas (HNSCC) totalling a number of 436 tests. The assay allows for the selective evaluation of chemoreactivity of both epithelial and stromal cells from a given biopsy specimen. *Results:* Reproducibility of single tests was demonstrated by parallel chemosensitivity tests with established cell lines performing under the same conditions as used for testing the biopsies. The selective analysis of epithelial and stromal cells revealed that stromal cells were equally or less sensitive to cytostatic drugs compared to the epithelial tumour cells in almost 90% of the tests. For comparative chemosensitivity tests, three separate biopsies from the same tumour were tested in parallel (n=10). Here, we consistently found the same chemosensitivities for epithelial cells from within one tumour. The chemosensitivity of stromal cells, however, varied widely among biopsies taken from different locations within the same tumour. *Conclusion:* Beside flavin-protecting culture conditions, a robust and cell type-specific evaluation of malignant cells from tumour tissues is required for reliable and meaningful chemosensitivity testing. According to our data, the cell type-specific chemosensitivity testing of a single tumour biopsy provides representative results for the epithelial cell population of a tumour.

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THE ROLE OF LYMPHANGIOGENESIS IN HUMAN NON-SMALL CELL LUNG CANCER

Balazs Dome

Department of Tumor Biology, National Koranyi Institute of Pulmonology, Piheno u. 1., Budapest, H-1529, Hungary

Although N status is a major determinant for the clinical management of non-small cell lung cancer (NSCLC), current strategies for lymph node (LN) staging is not sensitive enough, many cases are understaged, and we are unable to identify those patients who will ultimately have poor outcome. Consequently, there is a need for clinically useful approaches to better stratify patients with respect to the risk of LN metastasis and recurrence after surgery. Unfortunately, because no specific markers for lymphatic endothelium were available until recently, our knowledge of the lymphatic system of malignant tumors lags far behind that of the vascular system. However, the recent discovery of LYVE-1 and M2A antigen (D2-40) as specific markers for lymphatics has now provided tools for a detailed analysis of lymphangiogenesis. In a recent study, our group analyzed how NSCLC acquires its lymphatic network and investigated whether the extent of lymphangiogenesis might be related to the angiogenic phenotype (angiogenic *versus* nonangiogenic) and/or to the risk of lymph node metastasis and to patient survival, using

tumor samples obtained from NSCLC patients. For the first time in the literature, we demonstrated that lymphangiogenesis occurs exclusively in the angiogenic growth type of human NSCLCs, and that lymph vessel density is correlated to clinical behavior and to lymph node status only in this growth type of NSCLCs. However, this study also provided the first evidence that the risk of lymph node metastasis as well as a shorter survival is more likely to occur in the patient population with nonangiogenic tumors, and that these tumors mainly co-opt host tissue lymphatics during their growth, in contrast to most of the angiogenic ones, which expand with concomitant lymphangiogenesis. The current presentation provides an update on the biology of lymph vessels in human NSCLC, and explores the utility of lymphangiogenesis for pulmonary oncology.

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HEALTH-RELATED QUALITY OF LIFE: THE PARADIGM OF VACUUM-ASSISTED BREAST BIOPSY

Philip J. Domeyer

1st Department of surgery, School of Medicine, Athens University, Greece

Health-related quality of life (HRQoL) is an important parameter in cancer care and assessment of novel therapeutic strategies, as reflected upon numerous ongoing trials. Nevertheless, there is a relative scarcity of data regarding HRQoL in bioptical procedures, which are an indispensable part of early cancer diagnosis and subsequent management.

In this presentation the main point of focus is HRQoL in breast biopsy; vacuum-assisted breast biopsy (VABB) has been chosen as a recent, minimally invasive and reliable paradigm. VABB (11G needle) is capable of obtaining tissue for histopathological diagnosis of non-palpable mammographic lesions, so as to ensure early breast cancer diagnosis. Although its role is already well established, the impact of VABB on HRQoL has never been investigated.

The herein summarized research project has adopted two independent tools for the optimal assessment of HRQoL, namely SF-36 and EQ-5D. SF-36 comprises 36 items covering eight health dimensions. The EQ-5D is a short questionnaire which evaluates the patient's quality of life according to five dimensions, each one with three levels. EQ-5D also contains a visual analogue scale (VAS) on which patients rate their own health between 0 and 100. These questionnaires were completed by 128 patients, both in the morning of the VABB procedure and four days after VABB.

In the morning of VABB patients reported worse HRQoL scores in EQ-5D anxiety/depression and SF-36 general health subscales; a significant decline in EQ-5D usual activities and SF-36 role functioning-physical was observed four days after VABB.

The results clearly indicate for the first time a significant effect of VABB on HRQoL. Breast biopsy represents a significant event in a woman's life, with multidimensional physical, psychological and health care-related implications. Although the minimally invasive character of modern breast biopsy techniques might lead some health care providers to overlook its effect on HRQoL, measurement of the latter should be integrated in the global clinical assessment of patients.

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INFLUENCE OF GROWTH FACTORS ON THE FORMATION OF CD133-POSITIVE STEM CELLS FROM CD133-NEGATIVE POPULATIONS AND LOW PASSAGE HIGH-GRADE GLIOBLASTOMA MULTIFORME

Laura Donovan and Geoffrey J Pilkington

Cellular and Molecular Neuro-Oncology Research Group, Institute of Biomedical Sciences, School of Pharmacy and Biomedical Sciences, University of Portsmouth, St Michael's Building, White Swan Road, Portsmouth PO1 2DT, UK

Background: A small subset of cells displaying stem-like properties, with the ability to self-renew and sustain the growth of tumours has been identified in brain tumours. CD133 has been identified as a marker for a subpopulation of neural stem cells and facilitates an active role in local brain tumour cell invasion. Growth factors play a vital role in the modulation and behaviour of stem cells, in particular Human transforming growth factor β1 (TGFβ1), Epidermal growth factor (EGF) and basic-Fibroblast growth factor (bFGF/FGF-2). Initial research into the dependency of CD133-negative cells and low passage high-grade glioblastoma multiforme upon these particular growth factors will help to assess the formation and properties of CD133-positive stem cells. *Aim:* To isolate CD133-positive stem cell populations from glioblastoma multifoprme biopsies and to assess the role of growth factors in controlly CD133 positivity and biological activity of glioblastoma multiforme *in vitro*. Flow cytometry and immunocytochemistry will be used to characterise the varying levels of growth factor concentrations, using the appropriate immuno-markers CD133/1 and Ki-67. *Materials and Methods:* CD133-positive populations have been extracted by magnetic bead immuno-cell segregation (autoMACS™), from low-passage high-grade biopsy-derived glioblastoma multiforme cells and CD133-negative stem cell populations, and transformed into neurospheres by using stem cell defined growth media supplemented with the appropriate growth factors concentrations. The positive and negative isolated stem cell populations were characterised using flow

cytometry and immunocytochemistry with the CD133/1 antibody. *Results:* The influence of 10 ng/ml of TGFβ1, FGF-2 and EGF on CD133-positive stem cells was seen to effect cell proliferation rates, cell migration and CD133-positive expression. *Discussion:* The *in vitro* microenvironment dictates the behaviour of cultured neoplastic glia as well as influencing CD133 expression. Furthermore, studies are aimed at elucidating the precise role of multiple drug resistance of CD133-positive stem cells on the behaviour of glioma.

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COMBINATION THERAPY TARGETING G1 CYCLINS FOR PATIENTS WITH PREVIOUSLY TREATED ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC)

Konstantin H. Dragnev, James R. Rigas, Marc Seltzer, David Johnstone, William Nugent, Wendye M. DiSalvo, Sara Simeone and Ethan Dmitrovsky

Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756; Dartmouth Medical School, Hanover, NH 03755, USA

G1 cyclins are often aberrantly expressed in bronchial preneoplasia and lung cancer. This implicates these species as novel molecular pharmacological targets of several anticancer agents for lung cancer therapy and chemoprevention. We previously reported a targeted combination regimen that cooperatively affected D-type cyclins. A phase I trial of the EGFR tyrosine kinase inhibitor, erlotinib, and the rexinoid, bexarotene, in patients with advanced aerodigestive tract cancer showed minimal toxicities and preliminary evidence of clinical activity. Combining erlotinib with bexarotene also induced at least additive suppression of growth and cyclin D1 expression in human bronchial epithelial cells and in some lung cancer cell lines.

A phase II trial of erlotinib and bexarotene was conducted in patients with stage IV NSCLC. The primary objective was radiographic response rate; secondary objectives were survival, time to progression, toxicities, and correlation of early metabolic response by PET at 8-12 days and 2 months. Dosing was erlotinib 150 mg and bexarotene 400 mg/m^2 daily orally.

Forty-two patients were enrolled including 52% women and 62% with adenocarcinoma; the median age was 67 (46-77) years, 12% were current smokers and 17% were never smokers. The median number of prior therapies was 2 (range 0-5), 21% had had prior anti-EGFR therapy. Common toxicities were hypertriglyceridemia and skin rash. Grade 3 pulmonary hemorrhage (1), rash/mouth sores (1), cough (1), hypereosinophilic syndrome (1), and abdominal pain (1) led to treatment discontinuation. There were 2 objective partial responses; 7 patients had stable disease including one patient with prior gefitinib (35 weeks on study). Median time to progression was 7 weeks and median overall survival was 21 weeks (intent-to-treat). Decreased metabolic activity on PET imaging at 10 days was associated with radiographic response at 2 months. Correlation between the severity of hypertriglyceridemia and clinical outcome will be presented. The effect of this combined regimen on the levels of EGFR and/or cyclin D1 expression will be assessed in a proof-of-principle pilot study.

The combination of erlotinib and bexarotene is well tolerated and shows evidence of activity in heavily pretreated patients with NSCLC. These results implicate clinical benefit of dual targeting of EGFR and cyclin D1 in NSCLC. Future work should assess the effects of combinations of anticancer agents targeting cell cycle progression at G1.

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DNA-PKcs-PIDDOSOME: A NOVEL NUCLEAR CASPASE-2-ACTIVATING PROTEIN COMPLEX THAT FUNCTIONS IN DNA DAMAGE RESPONSE

Mingan Shi[1], Carolyn J. Vivian[1], Kyung-Jong Lee[2], Chunmin Ge[1], Keiko Morotomi-Yano[2], Shigeo Sato[1], Chieri Sato[1], Ruihong Zhu[1], Joshua Wunderlich[1], Selene K. Swanson[1], Jeffrey S. Haug[1], David J. Chen[2], Benjamin P. C. Chen[2], Michael P. Washburn[1], Laurence Florens[1] and Chunying Du[1]

[1]Stowers Institute for Medical Research, Kansas City, Missouri 64110;
[2]Division of Molecular Radiation Biology, University of Texas Southwestern Medical Center, Dallas, Texas, 75390, USA

Caspase-2 plays an apoptotic role in apoptosis. The identification of the protein complex PIDDosome has accelerated our understanding of caspase-2 activation. Caspase-2 is unique among all the mammalian caspases in that it is the only caspase that is present constitutively in the cell nucleus, in addition to other cellular compartments. However, the functional significance of this nuclear localization is unknown. We have found that DNA damage induced by γ-radiation triggers the phosphorylation of nuclear caspase-2, leading to its cleavage and activation. This phosphorylation is carried out by the nuclear serine/threonine protein kinase DNA-PKcs and is promoted by the death-domain protein PIDD within a large nuclear protein complex consisting of DNA-PKcs, PIDD, and caspase-2, which we have named DNA-PKcs-PIDDosome. Characterization of this DNA-PKcs-PIDDosome has revealed unexpected novel functions of it in DNA damage response pathway. Data will be presented to show the

molecular mechanism of this protein complex in the cellular response to DNA damage.

In conclusion, our data reveal a new role and a new activation mechanism of caspase-2. The ability of caspase-2 to participate in both pro-apoptotic and pro-survival processes means that caspase-2 may stand at the crossroads of a DNA-damage response network, coordinating apoptosis and cell survival to determine cell fate.

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THE ROLE OF THE *RETINOBLASTOMA* TUMOR SUPPRESSOR GENE IN CELL PROLIFERATION, DIFFERENTIATION, AND APOPTOSIS DURING NORMAL DEVELOPMENT AND IMPLICATIONS FOR TARGETED CANCER THERAPY

Wei Du

The University of Chicago, USA

The *retinoblastoma* gene (*Rb*) is a prototype tumor suppressor gene often mutated or inactivated in cancer. The Rb protein (pRb) regulates a variety of normal cellular processes including cell proliferation, differentiation, as well as apoptosis. Extensive studies have shown that pRb exerts its diverse functions by forming complexes with other proteins. Of the large number of proteins that can bind to pRb, the best studied are the E2F transcription factors, which are heterodimers composed of a subunit of the E2F family and a subunit of the DP family. In mammalian systems, there are eight E2F and three DP family members. While it is well established that E2F proteins mediate most, if not all, of the effects of *Rb* loss on cell proliferation and apoptosis, the mechanism by which *Rb* regulates cell differentiation is less clear. In addition to E2F, pRb also binds to a large number of other proteins including a number of transcription factors involved in the differentiation of specific cell types such as MyoD and C/EBP β. *In vitro* studies using cell culture systems have suggested that pRb directly interacted with and enhanced the activities of these differentiation promoting transcription factors to promote differentiation. However, the significance of these observations has not been demonstrated *in vivo* in animal models. The presence of large numbers of E2F proteins also makes it difficult to evaluate the contribution of E2F to the differentiation defects of *Rb* loss in mammalian systems.

The function and regulation of the Rb/E2F proteins are highly conserved and much simpler in *Drosophila*. We have used this model system to characterize the *in vivo* role of Rbf (fly Rb) in cell proliferation, differentiation, and apoptosis. Our analysis of developing fly tissues with *rbf* mutant clones revealed that the consequences of Rbf loss are distinct in different cell types, suggesting that additional signaling pathways or cell-intrinsic factors modulate the effect of Rbf removal. To identify genes that can preferentially affect the differentiation or death of *rbf* mutant cells, we carried out a genetic screen by generating double mutant clones. We found that mutation of *rno* leads to synergistic differentiation defects in conjunction with loss of Rbf. Further characterization revealed that the differentiation role of Rbf and Rno are mediated by their cooperative regulation of the Notch and EGFR signaling, and that this differentiation role of Rbf is E2F dependent. Characterization of the mutations that affected the apoptosis of *rbf* mutant cells revealed that *hid*, which is directly repressed by the Rbf/E2F repression complex, is a key player that mediates the apoptosis effect of *rbf* mutant cells. Genes and pathways that can modulate the activities of Hid can also modulate the apoptosis of *rbf* mutant cells. The conservation of these genes and pathways between flies and mammals suggest potential new approaches against cancer with *Rb* mutations.

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T-LYMPHOCYTE CYTOKINE PRODUCTION PATTERN OF THYROID CARCINOMA *VERSUS* NODULAR THYROID DISEASE

H. Duan, E. Maldonado-Gonzalez, M. Schuetz, K. Kaserer, B. Niederle, R. Dudczak, M. Willheim and G. Karanikas

Departments of Nuclear Medicine, Pathology, Surgery – Section of Endocrine Surgery and Pathophysiology, Medical University of Vienna, A-1090 Vienna, Austria

Aim: The balance of efficiency of immune reactions against transformed cells and impairment of the immune system caused by malignant cells plays a decisive role for the outcome of tumour disease. We evaluated of T-lymphocyte cytokine production pattern in patients with verified thyroid carcinomas and nodular thyroid diesease. *Materials and Methods:* Twenty-six patients (19 females, 7 male, aged 28-80 years) with nodular thyroid disease assigned for thyroidectomy were included in the present study. They were divided into two groups: Group I: 13 patients with histologically verified thyroid carcinoma (8 papillary, 2 papillary/follicular, 3 medullary carcinomas) and Group II: 13 patients with benign nodular thyroid. The control group (Group III) consisted of thirteen age-matched healthy volunteers free of thyroid disease. All patients underwent measurement of intracellular cytokine detection in $CD4^+$ and $CD8^+$ T-cells of peripheral blood mononuclear cells (PBMC) by flow cytometry before undergoing surgery. *Results:* No significant differences were found in the cytokine production pattern between thyorid cancer and nodular thyroid disease

or the control group. On the other hand, benign nodular disease, in comparison to the control group, showed a significant increase of $CD8^+$ cells producing TNF-α, IFN-γ and IL2/IFN-γ. *Conclusion:* According to our data, no change in the cytokine production pattern can be detected in blood lymphocytes of patients with thyroid carcinoma. Remarkably, benign nodular disease showed increased production of cytokines associated with cellular immunity by cytotoxic T-cells. This suggests that the immune system responds to benign transformation of thyroid cells, a response that may be impaired by malignant cells.

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11β-HYDROXYLASE-INHIBITOR [^{18}F]FETO FOR VISUALIZATION/ VERIFICATION OF ADRENOCORTICAL TUMOUR MASSES IN POSITRON EMISSION TOMOGRAPHY

H. Duan, M. Schuetz, W. Wadsak, M. Mitterhauser, L. Key Mien, B. Niederle, A. Gessl, R. Dudczak, K. Kletter and G. Karanikas

Departments of Nuclear Medicine and Surgery – Section of Endocrine Surgery, Medical University of Vienna, A-1090 Vienna, Austria

We aimed to investigate the novel PET radiopharmaceutical [^{18}F]FETO, an 11β-hydroxylase-inhibitor, in patients with adrenocortical masses. Twenty-five patients (14 females and 11 males, aged 15-72 years) with adrenocortical lesions (n=22, verified by CT and/or MRI) or as follow-up after adrenocortical carcinoma (ACC, n=3) were included in the present study. Dynamic and whole body images were performed after administration of 370 MBq [^{18}F]FETO. Visual interpretation, as well as semi-quantitative analysis using standardised uptake values (SUVs) was conducted.

In 19 out of 25 patients, [^{18}F]FETO uptake was massive in adrenal lesions, pronounced in contralateral adrenals and moderate in liver. In 2/25 patients, FETO showed intense bilateral accumulation (CT/MRI negative), one with adrenogenital syndrome, the other with bilateral hyperplasia (histologically verified). In follow-up of the three patients with ACC, FETO revealed one liver metastasis of ACC (inconclusive in CT/MRI, verified by histology). A patient with an adrenocortical lesion verified by CT/MRI was true negative in the FETO PET scan (rhabdomyosarcoma, verified by histology). The median SUV of adrenal masses was 22.4 (range 8.3-41.1), of contralateral adrenocortex 15.8 (range 8.1-23.7), and of liver 2.9 (range 2.0-4.1). No significant difference was found between the median SUV of biochemically active (n=10, median SUV 21.0) and non-active adrenocortical lesions (n=11, median SUV 23.1).

Due to the intense uptake of [^{18}F]FETO in the adrenal masses, a clear tumour visualization was achieved. FETO is an excellent imaging tool for the visualization of benign adrenocortical diseases, as it visualizes adrenal adenomas as well as hyperplasia.

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^{18}F-FLUOROAZOMYCINARABINOSIDE (FAZA) IN PATIENTS WITH CERVICAL CANCER FOR DETECTION OF HYPOXIA AREAS IN POSITRON EMISSION TOMOGRAPHY – A PILOT STUDY

H. Duan, M. Schuetz, S. Kommata, D. Lukic, E. Maldonado-Gonzalez, P. Angelberger, R. Schoen, R. Dudczak, K. Kletter, B. Bachtiary and G. Karanikas

Departments of Nuclear Medicine and Radiotherapy/Radiobiology, Medical University of Vienna, and Austrian Research Centers Seibersdorf, Austria

Background: Hypoxia has emerged as an important factor in tumour biology and response to cancer treatment. ^{18}F-fluoroazomycinarabinoside (^{18}FAZA) was recently introduced as a novel hypoxia tracer. The aim of our study was to evaluate the potential value of FAZA in visualisation of tumour hypoxia and in the individual treatment planning of patients with cervical cancer. *Materials and Methods:* Twelve patients (mean age, 53 years) with cervical carcinoma (T2Nx or TxN1) were included in our study. In addition to their routine pre-therapeutical staging, ^{18}FAZA PET was performed before, during (short before brachytherapy) and three months after radio-chemotherapy. The patients were scanned subsequently by the administration of 370 MBq ^{18}FAZA (dynamic scan) and 1, and 2 hours afterwards (static scan). Any tumour visualisations by FAZA were judged as hypoxic area. Standardized uptake values (SUV) and ratios to normal tissue (muscle) were calculated. *Results:* In the initial static scan (before therapy), 5 out of 12 patients showed hypoxic areas in the tumour side (SUV range: 1.6-2.6; T/M ratio range: 1.2-3.6). No hypoxic areas were found in the remaining 7 patients nor in the initial nor in the follow-up scans. Four out of the 5 positive patients showed a decreased FAZA uptake during the therapy (short before brachytherapy) (SUV range: 1.4-1.9; T/M ratio range: 1.2-2.1) and a further reduced accumulation after the radio-chemotherapy (SUV range: 1.1-1.9; T/N ratio range: 1.0-2.3). One patient showed no change in hypoxic areas neither during the therapy nor in the follow up scan. In contrast to the other patients, this patient was a non-responder to radio-chemotherapy. *Discussion:* According to our preliminary results, ^{18}FAZA PET is able to visualise hypoxic tissue in cervical cancer. Following the time course of tumour oxygenation by FAZA scans as in our study could be a potential tool in treatment planning.

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ALKALINE SPHINGOMYELINASE (NPP7) AND ITS IMPLICATIONS IN INTESTINAL TUMORIGENESIS AND INFLAMMATION

Rui-Dong Duan

Biomedical Centre B11, Institution of Clinical Sciences, University of Lund, S-221 84 Lund, Sweden

Alkaline sphingomyelinase (Alk-SMase) is an enzyme expressed in the intestinal mucosa and human liver. It is an ectoenzyme localized on the surface of the cell plasma membrane and is released into the intestinal lumen and bile by both bile acids and trypsin. The enzyme belongs to nucleotide pyrophosphatase/phosphodiesterase (NPP) family with specific activities against phospholipids including sphingomyelin, platelet-activating factor (PAF), and lysophosphatidylcholine. The enzyme generates the antiproliferative molecule ceramide, inactivates PAF, and competes with phospholipase D and reduces the formation of lysophosphatidic acid. Expression of the enzyme is inhibited by a high fat diet and stimulated by water-soluble fiber psyllium and also by some anti-inflammatory drugs such as 5-ASA and ursodeoxycholic acid. The levels of the enzyme in the intestinal mucosa are positively correlated with caspase 3, the key enzyme responsible for apoptosis. *In vitro* studies showed that alk-SMase inhibits cell proliferation in a dose-dependent manner accompanied by a reduction of sphingomyelin and formation of ceramide. Development of human colonic adenocarcinoma is associated with a progressive reduction of alk-SMase activity. Such reductions have also been identified in human hepatic diseases such as primary sclerosing cholangitis and steatosis, which increase the risk of liver cancer. The results above indicate that alk-SMase may serve as a tumour suppressor in both colon and liver.

By analysing alk-SMase in cancer cell lines and biopsy samples, we identified two aberrant forms of alk-SMase mRNA in human colon and liver cancer. The formation of the aberrant forms are caused by a skipping of one exon at the splicing level, resulting in destruction of the substrate binding sites and total inactivation of the enzyme. The findings provide a molecular background for the reduced enzyme activity found previously in cancer tissues.

We have developed a method to express the recombinant alk-SMase with full activity in yeast cells. We are also able to increase alk-SMase activity 2- to 4-fold through a single site mutation based on a 3-D structure predicted by molecular modulation. In studies with a rat ulcerative colitis model induced by dextran sulfate sodium, we found that injection of the yeast-expressed human alk-SMase in the colon significantly reduced the inflammation score and the expression of TNF-alpha. Histological examination showed that alk-SMase protected the colonic membrane from inflammatory destruction.

In conclusion, alk-SMase may play important roles in protecting both colon and liver from tumorigenesis. The recombinant enzyme may be used as a protein therapeutic tool to replace the mutant enzyme in cancer and inflammatory conditions.

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BLOOD OUTGROWTH ENDOTHELIAL CELL-BASED DELIVERY OF CANCER GENE THERAPY

Arkadiusz Z. Dudek

Division of Hematology, Oncology and Transplantation, University of Minnesota, Minneapolis, MN, USA

Angiogenesis and postnatal vasculogenesis are two processes involved in the formation of new vessels, an essential requirement for tumor growth and metastasis. We isolated endothelial cells from human blood mononuclear cells by selective culture. These blood outgrowth cells express endothelial cell markers and respond correctly to functional assays. To evaluate the potential of blood outgrowth endothelial cells to construct functional vessels *in vivo*, NOD-SCID mice were implanted with Lewis lung carcinoma cells subcutaneously. Blood outgrowth endothelial cells were then injected through the tail vein. Initial distribution of these cells occurred throughout the lung, liver, spleen, and tumor vessels, but 48 hours after injection they were only found in liver and tumor tissue. By day 24, they could mostly be found in tumor vasculature and this tumor selectivity correlated with an increase in tumor vessel counts as compared with control injections. We engineered blood outgrowth endothelial cells to deliver an angiogenic inhibitor directly to tumor endothelium by transducing these cells with the gene for human endostatin, and the soluble receptor for vascular endothelial growth factor. These cells maintained an endothelial phenotype and decreased tumor vascularization and tumor volume in a subcutaneous murine tumor model, as well as in spontaneous orthotopic breast cancer and glioma models. We conclude that blood outgrowth endothelial cells have the potential for tumor-specific delivery of cancer gene therapy.

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LARGE MULTIVALENT IMMUNOGEN VACCINE IN PATIENTS WITH METASTATIC MELANOMA

Arkadiusz Z. Dudek

Division of Hematology, Oncology and Transplantation, University of Minnesota, Minneapolis, MN, USA

Metastatic melanoma is an incurable malignancy with a median survival of 6-8 months. While melanoma is refractory to most

chemotherapy drugs, its growth may be regulated by immune mechanisms as shown by the rare occurrence of spontaneous remissions, the presence of cytotoxic tumor infiltrating lymphocytes (TIL) in resected tumors, and the demonstration of TIL cytotoxicity toward autologous tumor cells *in vitro*. Notably, interferon (IFN)-α2b and interleukin (IL)-2 have demonstrated modest efficacy in the treatment of advanced melanoma. Phase II studies using biochemotherapy for the treatment of metastatic melanoma resulted in response rates of ~50%, median time to disease progression of 5 months and an extended median survival of approximately 12 months. These results are consistent with the notion that progression of melanoma can be affected by immune stimulation.

Therapeutic benefit has also been demonstrated recently for vaccines in metastatic melanoma. There are various strategies for development and administration of tumor vaccines including those generated from tumor-derived peptides, irradiated autologous tumor cells, allogeneic cell lysates, dendritic cells, and gene-modified tumor or dendritic cells. A novel approach for vaccine-induced augmentation of tumor-specific cytotoxic T lymphocyte (CTL) responses has been developed utilizing cell-sized (5-μm diameter) latex or silica microspheres that function as a solid support for tumor antigen presentation. These artificial antigen-presenting cells are termed large multivalent immunogen (LMI).

A phase II study of autologous LMI vaccine for the treatment of stage IV melanoma has been completed. Vaccine production involved isolating tumor cell membranes obtained from melanoma specimens which were then attached to microspheres. Eligible patients were randomly assigned to one of three treatment groups, in cohorts of three to ensure balanced samples between treatment groups in each stratum. Group 1 received LMI; Group 2 received cyclophosphamide and LMI; and Group 3 received cyclophosphamide, LMI, and IL-2. LMI were coated with autologous tumor cell membrane. Patients received LMI (1×10^7, 5–μm silica spheres) vaccination by intradermal injection beginning on day 1 and continuing at 4-week intervals until the supply of tissue for producing additional doses of LMI vaccine was depleted. Cyclophosphamide infusions at 300 mg/m^2/d were given intravenously 7 days before vaccine. IL-2 infusions at 1.75×10^6 IU/m^2/d were given subcutaneously 5 days after each LMI administration for 1 week (*i.e.*, days 6-12, 34- 40, *etc.*). This trial evaluated the safety of LMI. No grade 4 toxicities (by NCI CTCAE v 3.0) were observed in any arm. Patients with malignant melanoma had median overall survival time of 15.4 months (95%; CI>7.99) and median overall time to disease progression of 2.8 months (95% CI: 1.87, 6.25). One patient with melanoma had documented partial response (by RECIST criteria). Multiple regression analysis showed that the number of LMI doses was inversely correlated with risk of death. Based on our results that demonstrate the safety and tolerability of LMI vaccine, further development of this therapy in the allogeneic setting is now being performed in a randomized placebo-controlled study.

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EARLY PREDICTION OF OSTEOSARCOMA HISTOLOGIC RESPONSE BY 18F-FDG PET: PRECLINICAL EVALUATION IN AN ORTHOTOPIC RAT MODEL

Aurélie Dutour[2,5], Anne-Valérie Decouvelaere[4,5*], Jacques Monteil[6*], Raphaël Rousseau[1,2,3,5] and Perrine Marec-Berard[3,5]

[1]Université de Lyon, Lyon, F-69003;
[2]Inserm, U590, Lyon, F-69008;
[3]Institut d'Hématologie-Oncologie Pédiatrique, Lyon, F-69008;
[4]Département d'Anatomopathologie et Cytologie, Lyon, F-69008;
[5]Centre Léon Bérard, Lyon, F-69008, 6 Service de Médecine Nucléaire, Centre Hospitalier Universitaire Dupuytren, Limoges, F-87000, France
*Both authors contributed equally to this work

The assessment of osteosarcoma response to neoadjuvant chemotherapy is performed after surgical resection of the primary tumor by histological analysis according to a method described by Huvos. The purpose of this study was to evaluate whether 18F-FDG PET could be a non-invasive surrogate of histopathological analysis and enable an early identification of response to neoadjuvant chemotherapy in osteosarcoma.

Rat orthototopic osteosarcoma model was used. Animals with well-established tumors were examined using 18F-FDG PET before being divided into a control and ifosfamide-treated group (n=10 animals/group). After two cycles of chemotherapy, all the animals were submitted to a second 18F-FDG PET exam. Tumors were then submitted to histological analysis. Image reconstructions of FDG PET were analysed semiquantitatively by using a volume of interest (VOI)-based method. Metabolic and histological responses to chemotherapy were compared. Histological analysis classified the rats from the control group as non-reponders, whereas animals from the ifosfamide-treated group were classified as partial (n=5) and good responders (n=5). Data analysis showed that the SUV Max value of the 18F-FDG PET performed after two cycles of chemotherapy correlated to the histological classification ($p<0.01$). We showed that an SUV Max <15 corresponded to a good responder, 15<SUV Max<20 to a partial responder and SUV Max>20 corresponded to non-responder. Moreover, analysis established that a 40% diminution of SUV Max between PET 1 and 2 is the cut-off value to distinguish a partial response from a good response to chemotherapy, with a specificity and sensitivity of 100%.

Semi-quantitative 18F-FDG PET using the SUV parameters seems to be able to predict the response to chemotherapy earlier than histological analysis.

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ROLE OF COLLAGEN- AND LAMININ-BINDING INTEGRINS IN LIVER METASTASIS

Johannes A. Eble

Center for Molecular Medicine, Department of Vascular Matrix Biology, Excellence Cluster Cardio-Pulmonary System, Frankfurt University Hospital, Frankfurt, Germany

Integrins, a versatile family of cell adhesion molecules, not only mediate the anchorage of cells within their surrounding extracellular matrix (ECM) but also act as important signal transduction molecules. Thus, they trigger a variety of matrix-induced cell functions, such as adhesion, force transmission, migration, differentiation, survival and apoptosis. In a physiological context, these integrin-mediated cell functions are vital. However, tumor cells also use integrins, albeit in an altered expression pattern, to disseminate from the primary tumor node through the interstitial stromal tissue and to penetrate the basement membrane, thus intra- and extravasating, and eventually colonizing distant organs.

The interstitial stroma tissue is rich in fibril-forming collagens, among them collagen I. The network-forming collagen IV and laminins are abundant constituents of the basement membrane. Hence, interactions of tumor cells with these matrix components *via* collagen- and laminin-binding integrins is of major importance in metastasis and may be a target for anticancer therapy.

The use of novel RGD-independent inhibitors of collagen- and laminin-binding integrins from snake venoms were tested for their ability to prevent tumor cells from extravasating liver sinusoids in an *in vivo* model. By comparison with *in vivo* models, the role of collagen- and laminin-binding integrins in the metastatic cascade could be defined. This may lead to new ways to curb metastasis.

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CYTOTOXICITY, DNA-BINDING PROPERTIES AND ANTIBACTERIAL ACTIVITY OF METAL-SPARFLOXACINATO COMPLEXES

Eleni K. Efthimiadou[1,2], Maria E. Katsarou[2], George Psomas[3] and Alexandra Karaliota[1]

[1]Department of Inorganic Chemistry, Faculty of Chemistry, National University of Athens, Panepistimioupoli Zographou, GR-15701 Athens;
[2]Institute of Physical Chemistry, NCSR ''Demokritos", GR-15310 Aghia Paraskevi Attikis;
[3]Department of General and Inorganic Chemistry, Faculty of Chemistry, P.O. Box 135, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece

Quinolones or quinolonecarboxylic acids are a group of synthetic antibacterial agents that effectively inhibit DNA replication and are commonly used as treatment for many infections (1). Sparfloxacin (=Hsf), a third-generation quinolone antimicrobial drug, is mainly used for the treatment of acute exacerbations of chronic bronchitis and community-acquired pneumonia (2). Sparfloxacin presents increased activity against Gram-positive species such as *Streptococcus pneumoniae* and *Staphylococci*. It has good bioavailability and its long half-life permits once-daily dosing, which may contribute to improved adherence to therapy and cost-effectiveness. DNA gyrase (topoisomerase II) and topoisomerase IV are targets of sparfloxacin against *Str. pneumoniae* and *Staph. aureus* (3, 4).

We present the structural and biological properties of the copper-sparfloxacinato complexes in the absence or presence of the N-donor heterocyclic ligands 2,2'-bipyridine or 1,10-phenanthroline as well as of the binary sparfloxacinato complexes with diverse transition metal ions. The antibacterial activity of the complexes against three microorganisms is presented and their DNA-binding behavior is evaluated. Additionally, some complexes have been tested as potential anticancer agents against human leukemia cell line HL-60 (peripheral blood human promyelocytic leukemia) and the cytotoxic effects are reported.

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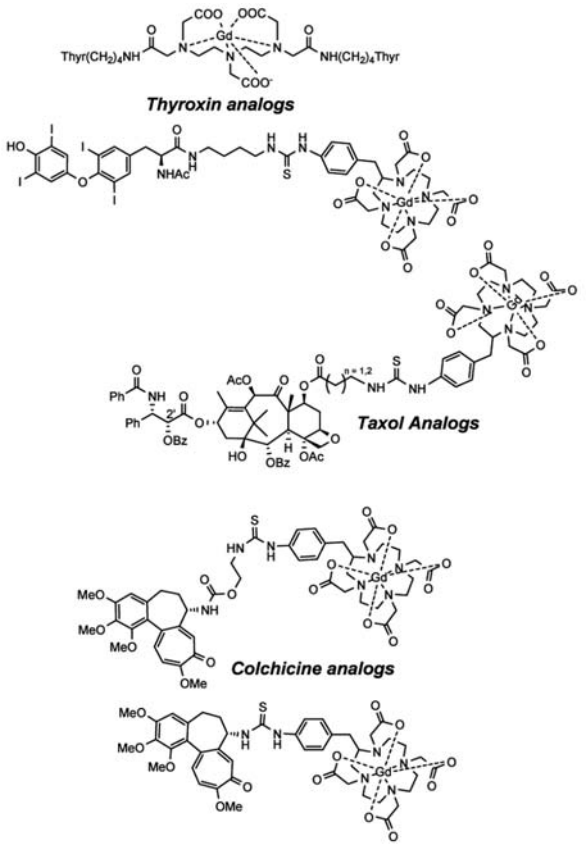
NEW CONTRAST AGENTS FOR MAGNETIC RESONANCE IMAGING TARGETING CANCER CELLS

Eleni Efthimiadou[1], Maria Katsarou[1], Michael Fardis[2], Christos Zikos[1], Emmanuel N. Pitsinos[1], Athanasios Kazantzis[1], Alexandra Karaliota[1], Leondios Leondiadis[3] and Dionisios Vourloumis[1]

[1]Chemical Biology, Institute of Physical Chemistry, NCSR "Demokritos", 15310 Aghia Paraskevi Attikis;
[2]Institute of Material Sciences, NCSR "Demokritos", 15310 Aghia Paraskevi Attikis;
[3]Institute of IRRP, Mass Spectrometry and Dioxin Analysis, NCSR "Demokritos", 15310 Aghia Paraskevi Attikis, Greece

Magnetic resonance imaging (MRI) is a non-invasive clinical imaging technique, which relies on the detection of NMR signals emitted by hydrogen protons in the body placed in a magnetic field. MRI contrast agent (CA) is a unique class of pharmaceuticals that enhances the image contrast between normal and diseased tissue, and indicates the status of organ function or blood flow after administration, by increasing the relaxation rates of water protons in the tissue, in which the agent accumulates. Contrast agents are being used in MRI since 1983 when the first injection of gadolinium (Gd) DTPA was performed in men (1). Since then CA have gained great acceptance. They function by shortening of T1-relaxation time, thereby increasing signal intensity (SI) (positive contrast enhancement).

Conceptually, antibodies or other tissue-specific molecules may be combined with paramagnetic centers to provide disease-specific MRI agents. The challenge with regard to delivering sufficient quantity of paramagnetic label is substantial. On that front, we have synthesized a series of gadolinium conjugates with Colchicine, Taxol™ and Thyroxin (Figure), known for their tubulin- and hormone receptor-binding properties respectively, targeting the discovery of novel, cancer specific CA. Cytotoxicity and relaxation time measurements have been performed for all these new complexes.



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MELANOMA CELLS REMEMBER THEIR ORIGINS

Ossia M. Eichhoff[1], Keith S. Hoek[1], Natalie C. Schlegel[1], Marie Zipser[1], Silvio Hemmi[2] and Reinhard Dummer[1]

1Department of Dermatology, University Hospital of Zurich, Zurich;
[2]Institute of Molecular Biology, University of Zurich, Zurich, Switzerland

Human primary and metastatic melanoma lesions frequently show heterogeneous staining pattern for melanocytic marker genes (*e.g.* Tyr, Melan-A). Cell cultures derived from metastases also show variable expression for these markers, and microarray analyses reveal two major expression signatures present in melanoma cell line libraries. One signature (proliferative) shows up-regulation of genes involved in melanocytic differentiation, while the other (invasive) shows up-regulation of factors involved in modifying the extracellular environment. These expression profiles correlate with phenotypic characteristics of proliferation, motility and growth factor sensitivity. We believe that expression of the melanocytic master-regulator Mitf is critical to the phenotype. Indeed, interruption of Mitf expression *via* siRNA reduced growth factor susceptibility by 60%, a characteristic of the invasive phenotype. We have derived a model in which melanoma cells may switch between proliferation and invasion to drive disease progression. Here we interpret a clinical case (thick primary and metastasis to the gall bladder) in the context of our phenotype switching model. Both primary and metastatic lesions show heterogeneity of staining for melanocytic markers, an aspect explained by our model. However, we also show evidence for microenvironment-dependent behaviour in the metastasis which demonstrates that programs reminiscent of healthy melanocytes may yet be recalled by melanoma cells. Melanoma cells encountering basal membrane structures in distal locations seek isolation from their peers and re-express dendritic structures. These are *in vivo* behaviours which are often reported to be lost during progression.

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CONCURRENT *VERSUS* SEQUENTIAL CHEMORADIOTHERAPY IN LIMITED-DISEASE SMALL-CELL LUNG CANCER: A RETROSPECTIVE COMPARATIVE STUDY

Sherif Y. El Sharouni[1], Henk B. Kal[1], Angeliqué Barten-Van Rijbroek[1], Henk Struikmans[2], Jan J. Battermann[1] and Franz M.N.H. Schramel[3]

[1]Department of Radiotherapy, University Medical Centre, Utrecht, Utrecht;
[2]Department of Radiotherapy, Leiden University Medical Centre, Leiden;
[3]Department of Pulmonary Diseases, St Antonius Hospital, Nieuwegein, the Netherlands

Introduction: Patients with limited-disease (LD) small-cell lung cancer (SCLC) received palliative treatment with chemotherapy (CT) or chemotherapy combined with radiotherapy. The treatment schemes with curative intention were sequential and concurrent chemoradiotherapy, both combined with prophylactic cranial irradiation (PCI). It is unclear which scheme is superior: sequential or concurrent chemoradiotherapy. *Methods:* Patient-, treatment- and outcome-related items were retrospectively assessed. Up till 2001, LD-SCLC patients received 4-5 cycles of cyclophosphamide, doxorubicin and etoposide. In cases of no complete response, either radiotherapy was given in 13 fractions of 3 Gy (CT-RT group) or no RT (CT group). After complete remission, radiotherapy was given in 16 fractions of 2.5 Gy, concurrently with PCI in 15 fractions of 2 Gy (SCT-RT group). From 2001, patients received 4-5 cycles of cisplatin and etoposide concurrently with radiotherapy in 25 fractions of 1.8 Gy. PCI was applied to patients with complete remission (CCT-RT group). Primary endpoints were median survival time (MST) and overall survival (OS); secondary endpoints included causes of death and frequency of metastases. *Results:* Median survival times of CT, CT-RT, SCT-RT and CCT-RT schemes were 8.1, 12.5, 14.0 and 21.8 months, and the 5-year OS was 3.5, 4.8, 10.5 and 26.9%, respectively. The cause of death of SCT-RT and CCT-RT patients was tumor related in 76.3% and 89.3% of the patients, respectively. Brain metastasis frequencies after PCI in SCT-RT and in CCT-RT patients were 16.4% and 8.7%, respectively. *Conclusion:* CCT-RT results in longer MST and higher OS than SCT-RT, CT or CT-RT.

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EFFECT OF NANO-FRUIT-CAFE ON THE EXPRESSION OF ONCOGENES AND TUMOR SUPPRESSOR GENES

László Szabó[1], István Kiss[2], Zsuzsanna Orsós[2] and István Ember[2]

[1]Crystal Institute, Eger;
[2]Institute of Preventive Medicine, Faculty of Medicine, Pécs University of Sciences, Pécs, Hungary

Fruit Café is a ground mixture of dried fruits and fruit seeds, intended for use in regular coffe-maker, to produce a high-flavonoid containing drink – a healthy alternative for coffee. Our earlier animal experiments demonstrated the cancer preventive effect of Fruit Café by using gene expressions as early biomarkers, and also in experiments with transplanted tumors.

The Fruit Café has been further developed by applying a nano-grinding technology, in order to reach a smaller particle size. This lead to an increased flavonoid content, and preparation of the drink became easier.

In the present study we examined the effect of this new Nano-Fruit-Café on the expression of oncogenes and tumor suppressor genes, in order to make a first evaluation of its possible cancer preventive characteristic.

Male CBA/Ca mice were intraperitoneally treated with Nano-Fruit-Café, with the body weight equivalent of the suggested human doses for everyday consumption. At different time points (5, 10, 15, 30 minutes, 1, 3, 6, 12, 18 and 24 hours) after the ip. injection the animals were overnarcotized, and RNA was isolated from their liver, spleen, kidneys, lung, thymus, bone marrow and lymph nodes. The RNA was blotted onto Hybond N+ membranes and hybridized with chemiluminescently labeled probes for c-myc, p53, Ha-ras, Ki-ras and Bcl-2 genes.

The expression pattern of the studied oncogenes and suppressor genes indicated a potential cancer chemopreventive effect for the new flavonoid containing drink. If these anticarcinogenic characteristics will be confirmed by using transplanted and chemically induced models, its widespread production and consumption might contribute to the chemopreventive strategies against cancer.

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CAN WE INCREASE CD34+ STEM CELL NUMBERS IN CIRCULATING PERIPHERAL BLOOD USING NATURAL COMPOUNDS IN ANIMALS?

I. Ember[1], L. Szabó[2], I. Kiss[1], T. Varjas[1] and Z. Gyöngyi[1]

[1]University of Pécs – Medical School, Institute of Public Health Medicine. Szigeti str. 12. Pécs;
[2]Crystal Institute. Grónay str. 12. Eger, Hungary

Stem cells were isolated from embryonic umbilical cord and various tissues (spleen, liver, lung, bone marrow, fat tissue) of CBA/Ca inbred H-2^k mice. Mice were treated with a mixture of natural components (Antrodia camphorate, Aphanizomenon, Fucoidan, Ganoderma lucidum, Zea mays, Lycium, cartilage of shark, hempseed and chlorophyll) to test its effect on stem cells. In every group, 6 male and female 4-6 week old mice were treated intraperitoneally with the mixture. After 1, 2, 3, 6, 18 and 24 h, CD34+ cells in peripheral blood were measured by flow cytometry and the

expression of several marker genes was also determined (p53, bcl-2, Ha- and K-ras, c-myc), in the various tissues. It was observed that stem cells from fat tissue and possibly from other sources may be activated.

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P38 MAP KINASE AS A POSSIBLE BIOMARKER OF EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS

István Ember[1], István Arany[2], Edit Nádasi[3], Tímea Varjas[1] and Zoltán Gyöngyi[1]

[1]University of Pécs Medical School Department of Public Health, Pécs, Hungary;
[2]Department of International Medicine Division of Nephrology, University of Arkansas for Medical Sciences, Markham Little Rock, AR, USA;
[3]Quintiles Magyarország, Budapest, Hungary

Health risk prediction and prevention of environmental diseases are highlighted topics of modern age. MAP kinases play a role in mitogenic and stress responses. In this manner, they could be appropriate candidates as biomarkers in the field of chemical carcinogenesis research. Polycyclic aromatic hydrocarbons (PAHs) are potent and abundant environmental carcinogens. Elevated expression of MAP (mitogen-activated protein) kinases may indicate tumour initiation, but also carcinogenic exposure. Some of them have also proven to be mutagenic. DMBA (7,12-dimethylbenz(alpha)anthracene) and 1-NP (1-nitropyrene) are important members of PAHs with carcinogenic potential. Since the family of MAP kinases play a role in diverse cellular events as proliferation and apoptosis, genes from that group could be candidates as biomarkers of exposure to chemical carcinogenes.

In the family of MAP kinases we focused on the activity of P38 protein. Rats, which are sensitive to chemical carcinogens, were administered with DMBA and 1-NP and the activity of P38 was detected in white blood cells by flow cytometry. Our results do not preclude that the activity of P38 could be a possible biomarker of exposure to chemical carcinogens, but further research is needed for verifying this aspect.

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THE CO-EXPRESSION OF JNK INTERACTING PROTEIN JIP-1 AND INSULIN LIKE FACTOR II CAN BE DISSOCIATED IN WILMS TUMOUR LINES BUT NOT IN PRIMARY WILMS TUMOURS

Wilhelm Engström

Division of Pathology, Department of Biomedical Sciences and Veterinary Public Health, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, PO Box 7028, 75007 Uppsala, Sweden

JNK interacting Protein 1 (JIP-1) is an important scaffolding protein in the JNK signalling pathway. It is also believed to play a role in the mediation of mitogenic messages from the plasma membrane to the cell interior. Previous studies suggest that the JIP-gene is co-regulated with the insulin like growth factor II (IGF II) gene, thereby contributing to the growth stimulatory effects of this potent growth factor. The striking co-expression of these two genes was found in murine fetuses as well as in primary human embryonic tumours. When ten primary Wilms Tumours were examined, the two genes showed a high degree of co-variation in the sense that high expression of IGF II was followed by high expression of JIP-1 and *vice versa*. However when the human Wilms Tumour cell line WCCS-1 was examined, a very modest intrinsic expression of IGF II was accompanied by a high expression of JIP-1. When exogenous IGF I was added, (which has previously been shown to induce apoptosis in this cell line), the JIP-expressiowas reduced to barely measureable levels. This data suggests that JIP-1 has a more complex role in the regulation of proliferation as well as programmed cell death.

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QUANTITATIVE MEMBRANE PROTEOMICS APPLYING NARROW RANGE PEPTIDE ISOELECTRIC FOCUSING FOR STUDIES OF SMALL CELL LUNG CANCER RESISTANCE MECHANISMS

Hanna Eriksson[1,2], Johan Lengqvist[1,3], Joel Hedlund[4], Kristina Uhlén[5], Lukas M Orre[1,3], Bengt Bjellqvist[5], Bengt Persson[4,6], Janne Lehtiö[1,3] and Per-Johan Jakobsson[1,2]

[1]Karolinska Biomics Center, Karolinska University Hospital, S-171 76 Stockholm;
[2]Department of Medicine, Rheumatology unit, Karolinska Institutet, S-171 76 Stockholm;
[3]Department of Oncology and Pathology, Karolinska Institutet, S-171 76 Stockholm;
[4]IFM Bioinformatics, Linköping University, S-581 83 Linköping;
[5]GE Healthcare Bio-Sciences AB, S-751 84 Uppsala;
[6]Department of Cell and Molecular Biology, Karolinska Institutet, S-171 77 Stockholm, Sweden

Drug resistance is often associated with up-regulation of membrane-associated drug efflux systems, and thus global membrane proteomics methods are valuable tools in the search for novel components of drug resistance phenotypes. Herein we have compared the microsomal proteome from the lung cancer cell line H69 and its isogenic Doxorubicin-resistant sub-cell line H69AR. The method used includes microsome preparation, iTRAQ labeling followed by narrow range peptide isoelectric focusing in an immobilized pH-gradient (IPG-IEF) and LC-MS/MS analysis. We demonstrate that the microsomal

preparation and iTRAQ labeling is reproducible regarding protein content and composition. The rationale using narrow range peptide IPG-IEF separation is demonstrated by its ability to: i) lowering the complexity of the sample by 2/3 while keeping high proteome coverage (96%), ii) providing high separation efficiency and iii) allowing for peptide validation and possibly identifications of post transcriptional modifications. After analyzing 1/5 of the IEF fractions (effective pH range 4.0-4.5), a total of 3704 proteins were identified, among which 527 were predicted to be membrane proteins. One of the proteins found to be differentially expressed was Serca 2, a calcium pump located in the ER membrane that potentially could result in changes of apoptotic response towards Doxorubicin.

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CURRENT STATUS OF FECAL TUMOR M2 PYRUVATE KINASE IN COLORECTAL CANCER SCREENING

N. Ewald[1], C. Tonus[2], K. Koss[3], R.M. McLoughlin[4] and P.D. Hardt[1]

[1]Third Medical Department, University Hospital of Giessen and Marburg, Giessen;
[2]Herz-Jesu Hospital, Fulda, Germany,
[3]Gastroenterology Department, Macclesfield District General Hospital, UK;
[4]Adelaide & Meath Hospital and Trinity College, Dublin, Ireland

Introduction: Colorectal cancer is a disease with major impact on public health and public health costs. Colonoscopy is currently supposed to be the best screening tool for colorectal cancer. However, the acceptance of this method is very poor, although it has been included in screening programs in the German health system since 2002. Thus, evaluation of additional screening tools seems to be of great interest. Recently, testing for fecal occult blood, genetic alterations or alterations in tumor metabolism (*e.g.* Tumor M2-PK) is under investigation. *Tumor M2 Pyruvate Kinase:* The use of M2-PK measurement in the feces has been reported in several studies to date. The data of these studies were analysed and critically reviewed. All available studies demonstrated a good performance of Tumor M2-PK in CRC screening, with an overall sensitivity ranging from 68.8% to 91.0%, and an overall specificity ranging from 71.9% to 100%. It might even be of some use in detection of larger adenomas, yet its sensitivity for adenomas is far lower (25.8-61.5%, depending on size). It should not be used for CRC screening in patients with inflammatory bowel disease since false-positive results can be expected in up to 90% (due to proliferation of epithelial cells and leucocytes in the inflammatory area). Since IBD patients, however, are subject to endoscopic surveillance anyway, they are not part of the population to be included in general CRC screening programs. Furthermore, a clear correlation between Tumor M2-PK levels in the feces and the stage of the tumor according to the Dukes' classification was demonstrated. Additionally it was noted that Tumor M2-PK level reduction is associated with successful surgical intervention. *Other non-invasive screening tests:* At the present time, the fecal occult blood test is still most commonly used as a non-invasive screening parameter for colorectal carcinoma. Yet fecal occult blood testing seems to be inferior, with a reported sensitivity of 40% for CRC and <20% for larger adenomas. Despite the improvement of diagnostic performance of FOBT obtained by immunological methods (iFOBT), one major limitation remains the fact that many carcinomas do not bleed at all or bleed only intermittently. Another available non-invasive screening method for CRC is testing for genetic alterations. Yet, the major shortcomings of this screening tool are its high costs, time consuming and tedious sample preparation, and the very limited handling and shipping time of the feces. *Conclusion:* Concerning handling, effectiveness and costs, fecal M2-PK therefore seems to be good for large-scale screening of colorectal carcinoma and should be recommended for inclusion in large-scale screening programs.

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THE RESPONSE OF THE CENTRAL NERVOUS SYSTEM TO IONIZING RADIATION: A CHALLENGE FOR RADIOBIOLOGY

A. Facoetti[1,2], R. Nano[2,3] and A. Ottolenghi[1,2]

[1]Department of Nuclear and Theoretical Physics, University of Pavia;
[2]INFN Pavia;
[3]Department of Animal Biology, University of Pavia, Italy

Ionizing radiation remains a major treatment modality for primary and metastatic tumours of the central nervous system (CNS) however the potential for injury to normal, non-targeted critical CNS structures limits the dose used in radiotherapy. Although the sequential histological changes associated with radiation injury have been well characterized, the cellular and biochemical processes responsible for the expression of radiation-induced CNS injury remain poorly defined, particularly at low doses. Much of the radiobiology efforts has been focused on the radiation response of the vascular endothelial cells and on cells of the oligodendrocyte lineage which remain the major cell type candidate for radiation induced demyelination and necrosis. However, not only the vasculature and glial progenitors are irradiated but also astrocytes, microglia, neurons and neural stem cells. Indeed the CNS is comprised of a number of disparate phenotypes networking to form a highly integrate system. Thus, the

implication of this nature of the CNS and its reliance on cell-cell interaction is that endocrine, paracrine, juxtacrine and contact-mediated processes should play a key role in the transmission of signals after irradiation and in the development of late effects. The purpose of this talk is to critically summarize the available information on the mechanisms responsible for the development of radiation-induced CNS injury and to suggest a framework for future research in this field. In addition, some experimental results on cell communication with normal and tumoural cells will be presented.

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ROLE OF CHROMATIN STRUCTURE IN TELOMERE MAINTENANCE

Zuzana Kunická[1,2], Eva Polanská[2], Martina Dvořáčková[2], Michal Štros[2] and Jiří Fajkus[1,2]

[1]Department of Functional Genomics and Proteomics, Institute of Experimental Biology, Faculty of Science, Masaryk University. CZ-62500 Brno;
[2]Institute of Biophysics, Czech Academy of Sciences, Královopolská 135, CZ-61265 Brno, Czech Republic

Nucleoprotein structures at chromosome ends, telomeres, protect chromosomes from degradation and fusions, and prevent natural chromosome ends from being recognized as chromosome breaks by repair mechanisms. The protective function of telomeres depends on maintenance of a certain minimum length of their DNA composed of (TTAGGG)n repeats (in vertebrates) and on telomere-associated proteins, which together constitute protective (capping) structures on telomeric DNA. Replicative shortening of telomeres or dysfunction of associated proteins results in genome instability.

In human cells, telomere maintenance is typically achieved by telomerase, a nucleoprotein enzyme complex elongating telomeres by reverse-transcription mechanism. In the absence of telomerase, an alternative mechanism associated with homologous recombination can be activated, which is called ALT (alternative lengthening of telomeres).

Since the major part of the telomere is folded in nucleosomes, forming a specific heterochromatin structure, epigenetic mechanisms can be involved in maintenance of telomere length homeostasis. Indeed, recent experimental studies have shown telomere length changes induced by changed levels of heterochromatin marks (including DNA methylation, H3K9me3 and H4K20me3). Telomere function is thus apparently tightly linked to chromatin architecture, and analysis of the contribution of other factors involved in chromatin dynamics is of special interest to current telomere biology.

Many of the chromatin structural changes are mediated by the large and diverse superfamily of HMG (high mobility group) proteins, including the HMGB-type proteins. In our study, we analyze the effect of a deficiency of HMGB1 protein in mouse embryonic fibroblasts (MEFs) derived from normal and HMGB1$^{-/-}$ knockout mice. We report a significantly lower telomerase activity, changes in telomere lengths, and increased occurrence of cytogenetic abnormalities in HMGB1$^{-/-}$ MEFs relative to the parental cell lines. Analyses of molecular causes of these effects identified HMGB1 as a telomerase-interacting factor.

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GASTRITIS OLGA STAGING AND GASTRIC CANCER SECONDARY PREVENTION

Matteo Fassan[1], Alberto Meggio[2], Gianmaria Pennelli[1,4], Claudia Mescoli[1], David Y. Graham[3] and Massimo Rugge[1,4]

[1]Department of Medical Diagnostic Sciences and Special Therapies (Pathology Section), University of Padova;
[2]Department of Gastroenterology, Rovereto Hospital, Italy;
[3]Digestive Disease Division, Veterans Affairs Medical Center and Baylor College of Medicine, Houston, Texas, USA;
[4]IOV-IRCCS, Padova, Italy

Objectives: Atrophic gastritis (mainly resulting from long-standing *Helicobacter pylori* infection) is a major risk factor for (intestinal-type) gastric cancer development. The extent/topography of atrophic changes significantly correlate with the degree of cancer risk. We previously proposed a new system for reporting gastritis in terms of staging (the OLGA staging system). Gastritis staging arranges the histological phenotypes of gastritis along a scale of progressively increasing gastric cancer risk, from the lowest (stage 0) to the highest (stage IV). In this study, we first tested the OLGA staging system in populations with different cancer risk. Secondly, we validated the staging system in a prospective cross-sectional study considering a large series of Italian patients. *Materials and Methods:* Mapped gastric biopsies were obtained from 755 dyspeptic adults, from 8 worldwide geographic areas (484 Italian) with different gastric cancer risk. Gastric atrophy was assessed according to internationally validated criteria. Gastric stage was established according to the OLGA staging system. Results were presented as stage (including antral and corpus atrophy scores) and *Helicobacter pilory* status. *Results:* The most prevalent gastritis stages were 0 to II. In populations at different cancer risk, the gastritis

OLGA stage mirrored the gastric cancer incidence. *Helicobacter pilory* infection was associated with a higher gastritis stage incidence. In the Italian series, benign conditions (including duodenal ulcers) consistently clustered in stages 0-II, whereas all neoplastic (invasive and non-invasive) lesions clustered in stages III-IV. *Conclusion:* OLGA gastritis staging, combined with *Helicobacter pilory* status, provided clinically relevant information on the overall status of the gastric mucosa with implications for prognosis, therapy and management.

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EVOLUTIONARY CONSERVATION OF METALLOPANSTIMULIN-1 (S27E RIBOSOMAL PROTEIN) SHOWS A KEY ROLE IN GROWTH REGULATION IN ARCHEA AND CARCINOGENESIS IN EUKARYOTIC CELLS

J. Alberto Fernandez-Pol

Metalloproteomics, Chesterfield, MO, USA

When the function of a protein serves a survival purpose, evolutionary laws of nature, conserves such protein, in phylogenetically related genus and species. Metallopanstimulin-1(MPS-1; S27E) Ribosomal Protein (RP), is involved in growth regulation, and carcinogenesis and conserved through evolution. Berthon *et al*. (2008) have shown in Archaea by genomic context analysis, a conserved cluster of genes coding for proteins involved in translation and ribosome biogenesis. These cluster of RP are *S27E (MPS-1), L44E, aIF-2 alpha* and *Nop10*, and are systematically contiguous to the group of genes coding for *PCNA, PriS,* and *Gins15*, which are involved in DNA replication. The two distinct sets of genes are involved in translation and ribosome biogenesis (Ribosomal cluster) and DNA replication (Replication cluster). Fernandez-Pol *et al*. (J Biol Chem & Mol Biol, 1993) was first to describe the gene encoding MPS-1 (S27E) and subsequently have shown that MPS-1 is overexpressed in many human cancer tissues. MPS-1 is being used as a target for some novel cancer therapies; aim to eject the zinc from the zinc finger motif of the MPS-1 protein, thus yielding it inactive. These and other therapies have shown promise for the treatment of cancer in humans, for example gastric cancer (Wang *et al*. 2006) and in lymphomas (Fernandez-Pol, 2001). Revenkova *et al*. (1999) found unexpected functions of MPS-1 RP in genotoxic stress responses in plants. These results indicate that an isoform of RP S27E (mutant MPS-1/ARS27E) is dispensable for the function of ribosomes, but is required for the elimination of damaged mRNA transcripts after exposure to chemical carcinogens or UV irradiation. MPS-1 is a RP with extra-ribosomal functions such as DNA repair and transcription. The results obtained with archaea (Berthon *et al*., 2008) and the functions of MPS-1 and other RP proteins studied in this laboratory indicate a previously unrecognized regulatory network coupling DNA replication, DNA repair, DNA transcription, translation and biogenesis of ribosomes that exist in both *Archaea* and *Eukarya*. Overexpression of RP genes observed in cancer is the result of increased translation of individual mRNAs rather than in up-regulation of global protein synthesis. We show that in association with increases in MPS-1 after growth factor stimulation, there is an increase of numerous ribosomal proteins as well as initiation factors (IF). Elimination of MPS-1 in cancer cells in eukaryotic animal and plant cells by novel and specific therapies may result in the cure or control of most cancers in animals, humans and plants.

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CHEMOSENSITIZING EFFECT OF NORDIHYDROGUAIARETIC ACID (NDGA) AND ITS TETRA ACETYLATED DERIVATIVE (NDGATA) ON PARENTAL AND MULTIRESISTANT TA3 MOUSE MAMMARY ADENOCARCINOMA CELL LINES

Jorge Ferreira[1], Mario Pavani[1], Fabián Jaña[1], Paula Burgos[1], Antonio Morello[1], Juan Diego Maya[1], Mario Faúndez[1], Américo López[1], Alfredo De Ioannes[2] and María Inés Becker[2]

[1]Programa de Farmacología Molecular y Clínica, ICBM, Facultad de Medicina, Universidad de Chile, Independencia 1027 Casilla 70086, Santiago 7;
[2]Departamento de Investigación y Desarrollo, Biosonda S.A., Eduardo Castillo Velasco 2902, Ñuñoa-Santiago, Chile

The clinical utility of several chemotherapeutic agents often limited by development of drug resistance, which is found in the multidrug-resistance (MDR) phenotype. It is frequently associated with overexpression of various ATP-binding cassette transporters which operate as drug-efflux pumps in MDR cells. The identification of agents able to synergistically modulate antineoplastic activity may be useful in overcoming resistance at non-toxic doses, especially those capable of circumventing cross-resistance to a large number of unrelated antineoplastic agents. Among these chemosensitizers, NDGA and its derivatives may be a class of great interest. Both NDGA and NDGATA can modulate the MDR phenotype, provoking selective induction of mitochondrial dysfunctions. Although both NDGA and NDGATA inhibited tumour growth of TA3 and TA3-MTX-R cell lines, they also strongly enhanced the toxicity of doxorubicin, cisplatin and methotrexate in a dose-dependent manner, with a more evident effect in the TA3-MTX-R cells than in the parental cells. NDGATA was more effective than NDGA. Analysis of the data by the isobole method showed that the combination of a chemotherapeutic agent with either NDGA or NDGATA

produced synergistic antiproliferative activities in both cell lines. The combination of NDGATA and DOX strongly reduced the tumor growth rate in mice and was the best treatment against the sensitive TA3 cell line and the resistant variant TA3-MTX-R. The inhibitory activity of this combination on the rates of solid tumor growth appeared to be synergistic. The combination of NDGATA and DOX did not prolong median survival time of mice with TA3 or TA3-MTX-R cells, but it rendered 30% of the mice with TA3 or TA3-MTX-R cells tumor free. On the other hand, NDGA and NDGATA inhibited primarily mitochondrial electron flow; therefore, transmembrane potential decreased and mitochondrial ATP synthesis was interrupted. They also increased the accumulation of doxorubicin and inhibited the efflux of rhodamine 123 from both the parental TA3 cell line, and from the resistant, TA3-MTX-R cell line. In addition, mitochondrial permeability transition pore was opened, cytochrome *c* was released and an increase of caspase 3 activity was detected. Consequently, cell viability and growth rate also decreased. In conclusion, NDGA and NDGATA are selective cytotoxic compounds to tumour cells. They inhibited the production of energy necessary for ATP-dependent transporter function, representing a new class of compounds that could be exploited for use in malignancies that display the phenomenon of MDR.

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MACROCYCLIC DITERPENES AS LEAD COMPOUNDS FOR THE DEVELOPMENT OF P-GLYCOPROTEIN MODULATORS IN MULTIDRUG-RESISTANT CANCER CELLS

Maria-José U. Ferreira[1], Noélia Duarte[1], Anett Járdánházy[2], Ana-Margarida Madureira[1], Cátia Ramalhete[1], Imre Ocsovszki[3] and Joseph Molnar[2]

[1]Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal;
[2]Department of Medical Microbiology, Faculty of Medicine, University of Szeged, Szeged;
[3]Institute of Biochemistry, University of Szeged, Szeged, Hungary

Chemotherapy remains the treatment of choice in many malignant diseases. However, the emergence of resistance to anticancer drugs (MDR) has made many of the available anticancer drugs ineffective. The most significant mechanism of MDR is that resulting from the overexpression of P-glycoprotein (Pgp), a member of ABC transporters that decreases the intracellular concentration of chemotherapeutics. Therefore, when used in combination, MDR modulators or inhibitors may restore the cytotoxicity of the anticancer drugs against MDR tumour cells.

In our search for anticancer agents from *Euphorbia* species, traditionally used to treat cancer, we have isolated and characterized, by spectroscopic methods, several macrocyclic lathyrane and jatrophane diterpenes, which were evaluated as Pgp modulators.

The ability of compounds to reduce the transport activity of Pgp on the L5178 mouse lymphoma cell line containing the human MDR1 gene was studied by flow-cytometry. The reversal of MDR was investigated by measuring the rhodamine-123 accumulation, a fluorescent substrate analogue of doxorubicin, in cancer cells. Verapamil was applied as a positive control.

In order to obtain evidence for synergistic interactions, the *in vitro* antiproliferative effects of some resistance modifiers were studied in combination with doxorubicine/epirubicine, on human *MDR1* gene-transfected mouse lymphoma cells, using the checkerboard microplate method.

The majority of the macrocyclic diterpenes tested showed to be very strong modulators of Pgp. The results obtained highlighted the importance of the involvement of ring A of lathyranes and jatrophanes in the modulation of Pgp. The importance of general requirements, such as lipophilicity and the potential ability to form H-bonds, was also corroborated by these results. All the diterpenes assayed for their antiproliferative effects in combination with doxorubicine/epirubicine, have shown synergistic interaction with the antitumour drug.

According to these results, macrocyclic diterpenes may be valuable as lead compounds for the development of Pgp inhibitors in different multidrug-resistant cancer cells.

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ANTINEOPLASIC ACTIVITY OF PHENOLIC COMPOUNDS AGAINST SENSITIVE AND RESISTANT HUMAN CANCER CELLS

Noélia Duarte[1], Hermann Lage[2] and Maria-José U. Ferreira[1]

[1]Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal;
[2]Charité Campus Mitte, Institute of Pathology, Berlin, Germany

Multidrug resistance (MDR) is one of the major problems in clinical oncology. This phenomenon is due to various biochemical mechanisms including overexpression of the well-known ABC transporter MDR1/P-gp. One possible approach to overcome MDR is to find out new anticancer drugs without cross resistance in cancer cells exhibiting a multidrug-resistant phenotype. The aim of this work was to analyze the antineoplasic activity of several phenolic

compounds isolated from *Euphorbia* species. The tested compounds, including stilbenes, flavanoids and coumarins, were investigated for their potential antiproliferative activity in several human cancer cell lines derived from three different tumour entities: gastric (EPG85-257), pancreatic (EPP85-181) and colon (HT-29) cancer cells. Furthermore, in each case, two different multidrug-resistant variants of these cells were also investigated: cell lines with a classical MDR phenotype (associated with the overexpression of MDR1/P-gp) and cell lines with an atypical MDR phenotype (no enhanced expression of MDR1/P-gp). For assessment of cytotoxicity of the tested compounds, the IC_{50} values of each agent were determined by proliferation assays in each of the different cell variants. The etoposide-specific IC_{50} values were measured as positive control for maintenance of the drug-resistant phenotype. Relative resistance (RR) values were also determined as the relation between the IC_{50} of the resistant cell line and the IC_{50} of the parental drug-sensitive cell line. In parental drug sensitive cell lines, all the tested compounds showed a weak antiproliferative effect. However, most of the multidrug-resistant cancer sublines showed increased sensitivities to the studied compounds when compared to the parental sublines. One flavanoid was found to be highly effective against the atypical MDR subline of gastric carcinoma, in which the flavanoid was 15-fold more effective than in parental "drug-sensitive" cells. The results obtained indicate that some of the phenolic compounds tested may be interesting for the development of new drugs against resistant human cancer cells.

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INVESTIGATION OF CHROMOSOMAL GAINS AND LOSSES IN CHAGASIC MEGAESOPHAGUS BY FLUORESCENCE IN SITU HYBRIDIZATION

Marilanda Ferreira Bellini[1,2], Patrícia Maluf Cury[3], Marileila Varella Garcia[2] and Ana Elizabete Silva[1]

[1]Department of Biology, UNESP, São Paulo State University, Campus São José do Rio Preto, SP, Brazil;
[2]Medicine/Medical Oncology, University of Colorado, Aurora, Colorado, USA;
[3]Hospital de Base/FAMERP – Medicine School, São José do Rio Preto, SP, Brazil

Chagasic megaesophagus is a dilation of the esophagus caused by the impact of the protozoa *Trypanosoma cruzi* in the mioenteric plexus. One of the most serious complications of megaesophagus is the increased risk (3%-8%) to develop esophageal squamous cell carcinoma, ESCC. While numerous genetic alterations have been reported in the initial and advanced steps of esophageal carcinogenesis, studies in chagasic megaesophagus are scarce, and this investigation aimed to use FISH technology to identify the genomic status in chagasic megaesophagus of genes showing recurrent imbalances in ESCC. This study cohort included 40 patients with diagnosis of chagasic megaesophagus. FISH probes were developed for the genes *FGFR1, PIK3CA, TP63, YES1* and *NCOA3*. Commercial probes were used for *EGFR*/CEP7, and *c-MYC*. Dual-color FISH assays were performed in formalin-fixed, paraffin-embedded tissue sectioned at 4 um. The analyses were carried out using single and dual band pass interference filters. For each specimen, signals were scored in 100 epithelial nuclei (50 in superficial layer – S, and 50 in basal layer – B). Descriptive statistics were calculated and the range of the mean copy number per cell was 1.58-1.94 (S) and 1.60-2.22 (B) for *FGFR1*, 1.56-1.96 (S) and 1.60-1.90 (B) for *PIK3CA*, 1.58-1.92 (S) and 1.60-1.90 (B) for *TP63*, 1.40-1.84 (S) and 1.28-1.80 (B) for *YES1*, 1.44-1.84 (S) and 1.44-1.76 (B) for *NCOA3*, 1.28-1.80 (S) and 1.18-1.80 (B) for *EGFR*, 1.22-1.74 (S) and 1.30-1.64 (B) for CEP7, 1.56-1.94 (S) and 1.60-1.84 (B) for *MYC*. The scoring was performed in both histological layers to check for differences between proliferative activity, since the basal cells have higher level of proliferative activity; however no difference was observed. Despite the involvement of genomic imbalances in esophageal carcinogenesis, none of the above genes were found unbalanced in chagasic megaesophagus.

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MOLECULAR CYTOGENETICS, MUTATIONS AND IMMUNOHISTOCHEMICAL STUDIES OF *P16* AND *FHIT* IN CHAGASIC MEGAESOPHAGUS

Marilanda Ferreira Bellini[1,2], Fernanda da Silva Manoel-Caetano[1], Patrícia Maluf Cury[3], Henrique de Olveira[3], Kenji Miyazaki[3], Marileila Varella Garcia[2] and Ana Elizabete Silva[1]

[1]Department of Biology, UNESP, São Paulo State University, Campus São José do Rio Preto, SP, Brazil;
[2]Medicine/Medical Oncology, University of Colorado, Aurora, Colorado, USA;
[3]Hospital de Base/FAMERP – Medicine School, São José do Rio Preto, SP, Brazil

Chagasic megaesophagus is a dilation of the esophagus caused by the impact of the protozoa *Trypanosoma cruzi*, etiological agent of Chagas disease. One of the most serious complications of megaesophagus is the increased risk (3%-8%) to develop esophageal squamous cell carcinoma, ESCC. While numerous genetic alterations have been reported in the initial and advanced steps of esophageal carcinogenesis, studies in chagasic megaesophagus are scarce. So, this investigation aimed to use FISH, PCR-SSCP/sequencing of DNA and immunohistochemistry technologies to identify the

genomic and protein status of *P16* and *FHIT* in chagasic megaesophagus. This study cohort included 20 esophageal biopsies of patients with diagnosis of chagasic megaesophagus and 10 normal esophageal mucosa biopsies. For FISH, signals were scored in 100 epithelial nuclei (50 in superficial layer – S, and 50 in basal layer – B). Descriptive statistics were calculated and the range of the mean copy number per cell was 1.28-1.74 (S) and 1.30-1.82 (B) for *P16* and 1.58-1.92 (S) and 1.60-1.92 (B) for *FHIT*. The analysis of mutation in the exons 1 and 2 of *P16* and 5 and 7 of *FHIT* shown a silent mutation (polymorphism) at codon 88 (exon 7) in the *FHIT* gene in a sample of megaesophagus. Immunostaining for p16 (brown nuclear and cytoplasmic staining) and Fhit (brown cytoplasmic staining) proteins was graded by intensity of staining as negative *i.e.* (–) absence brown staining, or (+) weakly stained; or as positive *i.e.* (++) moderately stained and (+++) strongly stained. Positive scores (++/+++) correspond to normal protein expression, while negative immunostaining (–/+) is associated with loss of protein expression. Descriptive statistics, Kruskall-Wallis test with Dunn post-test was used to determined statistical significance. Despite the progressive loss of expression of p16 protein, none statistical difference was observed. The absence of stain was not observed for Fhit protein: the immunostaining was diffuse, ranging from weak to strongly stained. However, statistical significance was not found for Fhit expression. We can concluded the three techniques showed similar results, indicating that genetic alterations in *P16* and *FHIT* are not common events in chagasic megaesophagus, although they are related to ESCC development.

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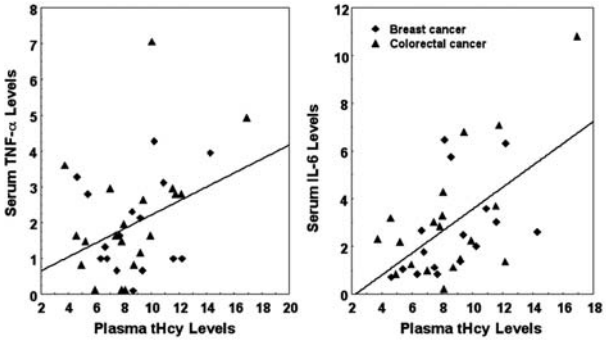
DETERMINANTS OF HOMOCYSTEINE LEVELS IN COLORECTAL AND BREAST CANCER PATIENTS

P. Ferroni[1], R. Palmirotta[1], F. Martini[1], F. Ciatti[1], S. Riondino[1], A. Magnapera[1], V. Sini[2], S. Mariotti[2], G. Del Monte[2], M. Roselli[2] and F. Guadagni[1]

[1]Department of Laboratory Medicine and Advanced Biotechnologies, IRCCS San Raffaele, Rome; [2]Medical Oncology, Department of Internal Medicine, University of Rome "Tor Vergata", Rome, Italy

Background: Methylation abnormalities appear to be important for the pathogenesis of many cancer types. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the homocysteine (Hcy) metabolism pathway and regulates the intracellular folate pool for synthesis and methylation of DNA. Accordingly, MTHFR 677TT variant increases Hcy concentration and reduces DNA methylation in cancer patients. However, Hcy metabolism is dependent not only on genetic, but also on acquired factors. Targeting TNF pathway can significantly decrease total Hcy (tHcy), suggesting a role for this cytokine in Hcy metabolism. Therefore, we analyzed the possible associations between tHcy levels and MTHFR polymorphisms or inflammatory markers in patients with breast or colorectal cancer. *Methods:* Forty-seven patients (15 males, mean age 59±14 years) with primary (n=35) or relapsing (n=12) breast (n=18) or colorectal cancer (n=29), treated at the Medical Oncology of "Tor Vergata" Clinical Center, were enrolled into the study. All patients were followed up for a median period of 14 months. Informed consent was obtained from all patients. MTHFR 677C→T and 1298A→C substitutions were analyzed by RT-PCR (Roche). Serum tHcy, high sensitive C-reactive protein (hsCRP), IL-6, TNF-alpha, fibrinogen and D-dimer levels were also analyzed. *Results:* MTHFR genotypes distribution was similar in patients compared to controls. Plasma tHcy ($p<0.05$), IL-6 ($p<0.05$), TNF-alpha ($p<0.05$) and D-dimer ($p<0.05$) levels were all significantly higher in patients compared to healthy controls. No differences were observed between breast and colorectal cancer patients for all laboratory variables. THcy levels significantly correlated with both IL-6 and TNF-alpha (Figure). Analysis of variance by Anova test showed that serum tHcy levels were not associated to either MTHFR 677C→T or 1298A→C. Multiple regression analysis including tHcy as the dependent variable and sex, age, diagnosis, metastasis, fibrinogen, D-dimer, hsCRP, IL-6, TNF and MTHFR polymorphisms as the predictor variables showed that metastatic disease (beta=0.50, $p<0.01$) and TNF (beta=0.34, $p<0.05$) were the only independent predictors of elevated tHcy levels. TNF, in turn, was significantly associated to the presence of lymph node involvement (beta=0.54, $p<0.005$). *Conclusion:* Our data indicate that the MTHFR polymorphisms do not significantly contribute to tHcy levels in breast and colorectal cancer, while we show some evidence that cancer-related inflammation may be associated with elevated tHcy levels, possibly involving a TNF-alpha mediated pathway.



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MILLIMETER WAVES EXPOSURE SET-UP FOR REAL-TIME MONITORING OF BIOLOGICAL PROCESSES AT A MOLECULAR LEVEL BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

L. Filippelli[1], A. Beneduci1, K. Cosentino[1], P.W. Westerman[2] and Giuseppe Chidichimo[1]

[1]Department of Chemistry, University of Calabria, Via P. Bucci, Cubo 17/D, Arcavacata di Rende (CS), Italy; 2Department of Biochemistry and Molecular pathology, NEOUCOM, Rootstown, Ohio, USA

Millimetre wave therapy (30-80 GHz) has been reported to significantly reduce *in vitro* cell proliferation of MCF-7 breast cancer cells as well as RPMI 7932 human melanoma cell line (1-3). Many experiments suggested that cell membranes might be one of the relevant targets of the radiation and studies on models showed that the water balance around the membrane is altered by exposing samples to low power millimetre waves (4). When we deal with the study of the interaction between millimeter wave and biological systems, one of the main challenges is to monitor relevant biological processes that characterize a system during its exposure to the above electromagnetic radiation. This condition should allow one to observe, in real-time, any significant variation of those properties and then to evaluate the biological effects induced by the radiation and its dependence on various exposure parameters such as frequency, power density and irradiation time, that might affect the response of the system. Nuclear magnetic resonance spectroscopy (NMR) is a very versatile technique for investigating, at a molecular level, several functional properties of various biological systems. Here we describe the experimental set-up used for the exposure of various biosystems to millimeter waves contextually to the acquisition of NMR spectra. The enormous potentiality of our method is underlined by showing some noticeable examples of its application, such as in the study of the metabolism of perfused living cells, or for the characterization of the structural and kinetic properties of bilayer membrane models. A brief discussion on millimeter wave-induced effects observed is also presented.

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NEW RESULTS ON BISPHOSPHONATES RELATED OSTEONECROSIS OF THE JAWS: TREATMENT COMPARISON AND EPIDEMIOLOGY

Olivier Filleul and Sven Saussez

Laboratory of anatomy, University of Mons-Hainaut, Pentagone 1B – Avenue du Champ de Mars, 6, B-7000 Mons, Belgium

Introduction: Bisphosphonate-related osteonecrosis of the jaws (BROJ) is a severe complication of bisphosphonate treatment. At this time, the best therapy as well as the epidemiology of this pathology are still unclear. *Methods:* A retrospective study of all cases of BROJ treated in four Belgian institutions was performed, aimed to compare medical to surgical treatment and find pronostic factors. *Results:* 34 cases were retrieved, of whom 88.5% were treated for disseminated cancers and 11.5% for osteoporosis. In our study, the most frequently used bisphosphonate was zoledronic acid (83%), either alone or in combination with pamidronate or ibandronate. 57% of patients were cured with only medical treatment, which was significantly different from the 20% obtained with surgical management (p=0.02). This study also revealed that lesions smaller than 1 cm had a better prognosis (p=0.0009). *Discussion:* Although limited and retrospective, this study indicates that current surgical procedures are not beneficial in management of BROJ. Further studies, especially prospective, will have to be conducted to propose better approaches. In our search for epidemiological and clinical data on this disease, we are conducting an exhaustive review of all described cases of BROJ, hoping this will lead to new enlightments on the pathophysiology and treatment of this condition. This study has already reached nearly 2000 cases.

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TAp63 IS A KEY REGULATOR OF DNA DAMAGE, GENOMIC STABILITY AND STEM CELL MAINTENANCE STEM CELL MAINTENANCE

Xiaohua Su[1], Min Soon Cho[1,2], Young Jin Gi[1], Yu-Li Lin[1], Wei X. Zhang[1] and Elsa R. Flores[1,2]

[1]The University of Texas M.D. Anderson Cancer Center, Department of Molecular and Cellular Oncology and [2]The University of Texas Graduate School of Biomedical Sciences, Houston, 77030, TX, USA

p63 is critical for the maintenance of epidermal stem cell proliferation, differentiation, and also plays a role in the suppression of tumorigenesis and metastasis. The existence of multiple isoforms (TA and ΔN) of *p63* with apparently opposing functions has complicated the study of *p63*. To begin to decipher the roles of these isoforms *in vivo*, we have generated *TAp63* conditional knockout mice (*TAp63*$^{fl/fl}$) using the cre-loxP system and intercrossed them with *zp3-cre* transgenic mice to generate *TAp63*$^{-/-}$ mice. Interestingly, some of the *TAp63*$^{-/-}$ mice have an early embryonic lethal phenotype at E6.5. The mice that survive to birth develop severe ulcerated wounds by the age of 1 to 3 months. These mice also exhibit signs of premature aging, increased DNA damage, and genomic instability in dermal and epidermal cells. These defects result from a hyperproliferation and subsequent premature depletion of stem cells involved in wound healing. We also found that levels of TAp63 are induced in the wound healing process. In addition to the striking role that *TAp63* plays in epidermal repair, we have found that mice deficient for this isoform are tumor prone. *TAp63* deletion in combination with p53 results in a highly metastatic tumor phenotype. These studies have unveiled previously unrecognized roles for *TAp63* in DNA damage, epithelial wound repair, and in cancer.

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ASSOCIATION OF PROSTATE-SPECIFIC ANTIGEN AND PROINFLAMMATORY CYTOKINES IN PATHOLOGICAL HUMAN PROSTATE (BENIGN HYPERPLASIA AND CARCINOMA): A STUDY OF TUNISSIAN PATIENTS

B. Fraile[1], Y. Bouraoui[1,2], Gonzalo Rodríguez-Berruguete[1], R. Oueslati[2], R. Paniagua[1] and M. Royuela[1]

[1]Department of Cell Biology and Genetics. University of Alcalá, E-28871. Alcalá de Henares, Madrid, Spain;
[2]Unit of Immunology and Microbiology Environnemental and Carcinogenesis IMEC, Faculty of Sciences of Bizerte 7021 Zarzouna, Tunisia

Aim: Serum prostate-specific antigen (PSA) level is more reflective of the presence of benign prostatic hyperplasia (BPH) than of the extent of cancer and, therefore, does not provide additional information. At present, several research groups are focusing on the search for new biochemical markers capable of predicting prostate cancer and prognosis. In addition, pro-inflammatory cytokines are related to the production of PSA and progression of prostate cancer The aim of this study was to relate the serum PSA levels with the expression of several pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) and their receptors in normal and pathological (hyperplasia and cancer) prostatic tissue to elucidate their possible role in tumor progression. We also discuss the possible use of these cytokines as potential therapeutics. *Methods:* The study was carried out in 5 normal, 25 benign prostatic hyperplastic (BPH) and 18 carcinomatous (PC) human prostates. Immunohistochemical and Western blot analysis were performed. Serum levels of PSA were assayed by IMMULITE autoanalyser. *Results:* The results most relevant showed that in BPH, IL-1α, IL-6 and TNF were only expressed in the patients included in the groups with PSA serum levels of 0-4 ng/ml PSA or 4-20 ng/ml PSA, but not in the group with PSA >20 ngml. In PC, these cytoines were only expressed in the patients included in the groups with PSA serum levels >4 ng/ml, increasing the expression when these patients were included in the group with most elevated PSA level (>20 ng/ml). *Conclusion:* In PC there was an association between the high expression of TNFα, IL-6, IL-1, elevated PSA serum levels and tumor progression. A better understanding of the biological mechanism and role played by the elevation of circulating PSA related with the tissue expression of these cytokines may possibly improve clinical management and provide new targets for therapy in these patients.

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CANCER PROGRESSION: IMMUNOHISTOCHEMICAL STUDY OF SURVIVIN AND XIAP IN NORMAL AND PATHOLOGICAL (BENIGN HYPERPLASTIC AND CANCER) HUMAN PROSTATE

B. Fraile[1], C. Chaves[1], Gonzalo Rodríguez-Berruguete[1], C. Nuñez[1], P. Martínez-Onsurbe[2], G. Olmedilla[2], F. Bethecourt[3], R. Cansino[3], R. Paniagua[3] and M. Royuela[1]

[1]Department of Cell Biology and Genetics, University of Alcalá;
[2]Department of Pathology, Príncipe de Asturias Hospital. Alcalá de Henares;
[3]Department of Urología del Hospital La Paz. Madrid, Spain

Aim: Inhibitor of apoptosis proteins (IAPs) is a gene family that plays an essential role in the negative regulation of apoptosis. The IAP family comprises eight proteins: survivin, XIAP (ILP-1), cIAP1, cIAP2, NAIP, ILP-2, apollon (BRUCE) and ML-IAP (LIVIN). The IAPs are potent inhibitors of caspases and inhibit apoptosis by a variety of stimuli (Deveraux, 1999). XIAP and survivin have been identified as the most potent inhibitors of caspases and apoptosis. The aim of this study was to elucidate the possible involvement of these IAPs in prostate cancer development and their role in the breakdown of the apoptosis-proliferation equilibrium.

Methods: Immunohistochemical and Western blot analyses were performed in 20 samples of normal prostate (NP), 35 samples of benign hyperplasia (BPH), and 93 samples of prostate cancer (PC) diagnosed with low (grades 1 to 6, 21 men), medium (grades 7-8, 51 men) and high (grades >8, 21 men) Gleason grades. *Results:* Immunoreaction to survivin was absent in normal prostates. Cytoplasmic immunoreaction to survivin in epithelial cells was observed in 9.1% of BPH patients and 19.35% of PC patients (optic density was increased with Gleason grade). Immunoreaction to XIAP was observed in the cytoplasm of epithelial cells in 20% of normal prostates, 27.27% of BPH patients and 32% of PC patients. Optical density was higher in BPH than in normal prostates, and even higher in PC, but no differences between Gleason groups were found. *Conclusion:* Survivin in several malignances has been associated with higher tumor grade, advanced disease stage, and as an unfavourable marker of disease progression. XIAP overexpression in tumor cells has been shown to cause an inhibitory effect on cell death. In this way, inhibition of these IAPs might be a possible target for PC treatment.

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INHIBITION OF HIF-2α PREVENTS TUMORIGENESIS

Aleksandra Franovic and Stephen Lee

Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5

The ability of cancer cells to proliferate autonomously is perhaps the most critical hallmark of malignancies. An example of how a genetic mutation can confer this oncogenic trait is the stabilization of hypoxia-inducible factor-2 alpha (HIF-2α) and its activation of the TGFα/EGFR growth circuit that drives VHL-loss clear cell renal carcinoma ($VHL^{-/-}$ RCC) tumor formation. HIF-2α is similarly expressed in the core of most solid tumors as a consequence of microenvironmental stressors such as hypoxia. This raises the possibility that the tumor microenvironment can promote the persistent proliferation of tumor cells under otherwise non-permissive conditions by stabilizing HIF-2α in a manner analogous to VHL-loss. Here, we show that silencing HIF-2α, but not the predominantly pro-angiogenic HIF-1α isoform, prevents *in vivo* proliferation and tumorigenesis of a panel of genetically diverse human cancers. Furthermore, treatment of xenograft tumors by intratumoral injection of siRNA against HIF-2α results in their regression, highlighting its importance in tumor maintenance. These results suggest that HIF-2α activation, as a result of genetic alterations or physiological stimuli, represents a common oncogenic event that is required for the establishment of an overt carcinoma. As such, we propose that targeting HIF-2α may be of broad clinical interest in the treatment of human cancer.

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ALLEVIATION OF SIDE-EFFECTS OF ANTICANCER THERAPEUTICS BY HMG-COA REDUCTASE INHIBITORS (STATINS)

Julia Damrot, Christian Ostrau, Johannes Hülsenbeck, Melanie Herzog, Tobias Nübel, Bernd Kaina and Gerhard Fritz

Institute of Toxicology, Johannes Gutenberg University of Mainz, Obere Zahlbacher Straße 67, D-55131 Mainz, Germany

Apart from their lipid-lowering activity, HMG-CoA reductase inhibitors (statins) also impact various genotoxic stress-induced signaling mechanims by inhibiting the function of regulatory proteins, in particular Ras and Rho GTPases. By this means, statins sensitize tumor cells to killing by anticancer drugs. Here we address the question whether statins are beneficial in anticancer therapy by alleviating side-effects of radiotherapy and the anticancer drug doxorubicin on normal tissue. To this end, we investigated the effect of lovastatin on ionizing radiation (IR)- and doxorubicin-induced signaling and apoptosis in primary human endothelial cells (HUVECs) *in vitro*. Moreover, initial *in vivo* studies were performed.

Low-dose pre-treatment with lovastatin protected HUVECs from IR- and doxorubicin-induced cytotoxicity, as measured by cell viability, cell proliferation and FACS-based apoptosis assays. IR- and doxorubicin-provoked increase in CD95L and CD95R mRNA expression was partially blocked by lovastatin. Activation of executor caspases was not detected 48-72 h after exposure, yet IR-stimulated apoptosis was blocked by the pan-caspase inhibitor Z-VAD. Examining the effect of lovastatin on DNA strand break induction (using the comet assay) and ATM/ATR-mediated H2AX phosphorylation (γ-H2AX), we found radioprotection by lovastatin to be independent of the formation and repair of DNA damage. In contrast, doxorubicin-triggered DNA strand break induction was attenuated by lovastatin, which was not due to alterations in doxorubicin uptake or efflux. Doxorubicin and IR-inducible DNA damage-related stress responses, including accumulation of p53 and p21 protein as well as activation of checkpoint kinase (Chk-1), stress kinases (SAPK/JNK) and NF-κB, were impaired upon statin pretreatment. Moreover, IR-induced NF-κB-dependent up-regulation of the cell adhesion molecule E-selectin was reduced by lovastatin.

Overall, the data show that the HMG-CoA reductase inhibitor lovastatin has pleiotropic inhibitory effects on IR- and doxorubicin-induced stress responses in HUVEC and eventually operates in an antiapoptotic manner. Preliminary *in vivo* data are in line with the *in vitro* results. Therefore, we suggest that lovastatin might be clinically useful in alleviating side-effects of IR and doxorubicin on normal tissue during tumor therapy.

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DEVELOPMENT OF A THREE-DIMENSIONAL (3D) ALL-HUMAN, *IN VITRO* MODEL OF THE BLOOD-BRAIN BARRIER FOR CANCER METASTASIS STUDIES USING ELECTRIC CELL-SUBSTRATE IMPEDANCE SENSING SYSTEM (ECIS) AND TRANSWELLS

K.E. Fry and G.J. Pilkington

Cellular and Molecular Neuro-oncology Research Group, Institute of Biomedical and Biomolecular Sciences, School of Pharmacy and Biomedical Sciences, University of Portsmouth, St Michael's Building, White Swan Road, Portsmouth PO1 2DT, UK

Background: Around 25% of cancers will spread to the brain by passing through the physical blood-brain barrier (B-BB) and thereby worsen prognosis. Melanoma, breast and lung cancer are among the most frequent primary tumours which metastasise to the brain. *In vitro* models of the B-BB generally utilise murine or porcine brain endothelium and rat astrocytes. In addition, these models are grown in foetal calf serum supplemented conditions which modify growth rates and cell adhesive properties. *Aim:* To develop a 3D *in vitro* model from human brain-derived cells under human supplementation for the study of the passage of cancer cells across the cellular B-BB using different techniques such as ECIS, Transwells and Flocel. *Materials and Methods:* The B-BB model is comprised of human astrocytes (CC-2565) with human cerebral microvascular endothelial cells (hCMEC/D3) immortalised with hTERT/SV40 LargeT antigen, under human serum supplementation. Non-small lung cancer, breast cancer and melanoma cells have been chosen to add to the model. All cells have been characterised with appropriate immuno markers using flow cytometry and immunocytochemistry while growth curves and adhesion properties have been established for the B-BB components. Electric cell-substrate impedance sensing system (ECIS) has been investigated along with co-culturing on Transwells. *Results:* Growth curves, antigenic expression, adhesive properties and growth on Transwells have been established. ECIS has demonstrated the potential of hCMEC/D3 to form a tight barrier and the difference extracellular matrices and conditioned media has on the impedance values. Cancer cells have also been assessed when added to the hCMEC/D3 monolayer. *Discussion:* We are currently assessing the model using a combination of live cell imaging, TIRF microscopy and confocal microscopy. The model has been developed using Transwells and on going ECIS experiments of co-culturing with CC-2565 will be explored. Flocel will be the next technique to be studied. We aim to identify discrete pathways underlying entry of various metastatic somatic cancer cells into the brain.

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QUADRUPLEX DNA STRUCTURES AS MODULATORS OF GENE EXPRESSION: MYOGENIC TRANSCRIPTION FACTORS INTERACT DIFFERENTIALLY WITH TETRAHELICAL FORMATIONS OF GENE PROMOTER SEQUENCES

Anat Yafe, Jeny Shklover, Shulamit Etzioni, Pnina Weisman-Shomer, Eyal Bengal and Michael Fry

Department of Biochemistry, Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, POB 9649 Bat Galim, Haifa 31096, Israel

B-DNA can be readily transformed into non-B-DNA conformations by positive or negative superhelical stresses, or by the action of specific proteins. Among non-B-DNA structures, tetraplex or quadruplex configurations of guanine-rich sequences are of growing interest. Tetrahelical structures in promoter sequences were implicated in the regulation of expression of multiple genes such as those that encode insulin, c-MYC, c-kit, bcl-2, VEGF and PDF-A.

We investigated the potential role of quadruplex structures of upstream sequences of muscle-specific genes in modulating the action of a family of myogenic regulatory factors (MRFs). These master transcription factors; MyoD, Myf5, MRF4 and myogenin, activate muscle gene expression by binding to conserved E-box elements, d(CANNTG), in their regulatory regions. We report that promoter and enhancer tracts of several muscle-specific genes contain, beside E-boxes, a high frequency of clusters of contiguous guanine residues that readily form hairpin and parallel-stranded unimolecular and bimolecular quadruplex structures. Further, homodimers of MyoD and MRF4 bind tetraplex structures of muscle-specific regulatory sequences much more tightly than their target E-box. By contrast, heterodimers of MyoD or MRF4 with an E47 protein form tighter complexes with E-box than with the tetraplex DNA structures. Although myogenin-E47 heterodimers also bind E-box preferentially, homodimers of myogenin bind quadruplex DNA weakly and non-preferentially. Structure–function analysis identified the E-box

and quadruplex DNA-binding sites in MyoD and Myogenin and established that the different basic domains of the two homodimeric proteins are the sole determinants of their different affinities for quadruplex DNA.

Based on our results, we offer models for the involvement of promoter quadruplex structures in the regulation of muscle-specific gene transcription.

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NOVEL STRATEGY OF ANTI-ANGIOGENIC THERAPY FOR UTERINE CERVICAL CANCER

Jiro Fujimoto

Department of Obstetrics and Gynecology, Graduate School of Gifu University School of Medicine, 1-1 Yanagido, Gifu City 501-1194, Japan

Angiogenesis is essential for development, growth and advancement of the primary tumor and especially its metastatic lesions. Angiogenic activity in tumors is related to the prognosis of most patients. Suppression of angiogenic potential in the tumors leads to inhibition of the primary tumor and its metastatic lesions. These facts prompted us to study the behaviour of overexpressed angiogenic factors and strategies for suppressing angiogenic potential.

The elevation of VEGF contributes to the relatively late advancement *via* angiogenic activity in advanced adenocarcinomas of the cervix (Brit J Cancer, 1999). Furthermore, VEGF associated with COX-2 works on advancement of uterine cervical cancer *via* angiogenesis, and long-term administration of COX-2 inhibitors might be effective on the suppression of regrowth or recurrence after intensive treatment for advanced uterine cervical cancer. IL-8 levels correlate with microvessel and infiltrated macrophage counts, and the localization of IL-8 is similar to that of CD68 specific to macrophages. The prognosis of patients with high IL-8 was extremely poor. Consequently, IL-8 can be regarded as a prognostic indicator as an angiogenic factor supplied from macrophages in uterine cervical cancer (Cancer Res, 2000). Thymidine phosphorylase (TP) has a wide range of expression and is highly expressed in uterine cervical cancer regardless of clinical stage. The prognosis of patients with high TP in primary tumors is worse than in those with low TP. Hence, it is apparent that TP in uterine cervical cancer plays a role of basic angiogenesis in all processes of advancement of uterine cervical cancer (Brit J Cancer, 1999). Furthermore, TP remarkably increased in 8 of 40 metastatic lymph node lesions of uterine cervical cancer, and the prognosis of the patients with high TP in metastatic lymph node lesions was extremely poor. Therefore, TP expressed in stromal cells appears to contribute to the advancement of metastatic lymph nodes after the establishment of metastasis, and is recognized as a prognostic indicator (Cancer Res, 1999). Incidentally, VEGF-C and osteopontin expressed in cancer cells directly contributes to lymph node metastasis in uterine cervical cancer (Brit J Cancer, 2004, Cancer Lett, 2007). In addition, serum TP is recognized as a novel tumor marker regardless of histopathological type of the uterine cervical cancer (Cancer Res, 2000). On the other hand, E 26 transcription-specific (ETS)-1 levels correlate with microvessel counts, and the localization of ETS-1 is similar to that of vascular endothelial cells in uterine cervical cancers. ETS-1 levels correlate with IL-8 and TP levels associated with HIF-1α levels. ETS-1 might work on angiogenesis as an angiogenic transcription factor and be a prognostic indicator in uterine cervical cancer. To avoid inducing alternative angiogenic pathways as a sort of tolerance to an angiogenic inhibitor, the simultaneous suppression of the main target angiogenic factors IL-8 and TP, and the transcription factor ETS-1 might be highly effective (Ann Oncol, 2002, Cancer Sci 2006). Furthermore, interferon-γ-inducible protein (IP)-10 inversely-correlates with microvessel density associated with VEGF, and might affect the suppression of angiogenesis in advancement. Therefore, IP-10 activation might be effective on the suppression of advanced uterine cervical cancers (Brit J Cancer, 2007). We are looking forward to proceeding with clinical trials of antiangiogenic agents in advansed-stage patients and after curative resection for uterine cervical cancer.

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THE FIRST *IN VIVO* EXPERIMENTS WITH THE COMBINATION OF TAXOL AND SILA-421, A NEW PROMISING MULTIDRUG RESISTANCE INHIBITOR IN HUMAN PANCREAS XENOGRAFTS

Andras Füredi[1], Attila Zalatnai[2] and Joseph Molnar[1]

[1]Institute of Medical Microbiology and Immunobiology, University of Szeged, Faculty of Medicine, H-6720 Szeged, Dóm tér 10.;
[2]1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Faculty of Medicine, H-1085 Budapest, Üllői út 26., Hungary

Cancer chemotherapy is one of the most important tools of the treatment of malignancies. The efficiency of chemotherapy is often reduced by the appearance of multidrug resistant cells in the tumour and during the treatment course these multidrug resistant cells overgrow sensitive cells due to a selective pressure of chemotherapy. One of the most frequent forms of MDR is an ABC transporter, the P-glycoprotein mediated resistance. This efflux pump extrudes chemotherapeutic agents from the cells, which is the major limitation of chemotherapy. Since inhibitors of MDR drug efflux can be promising agents to reverse the multidrug resistance, the effects of disiloxans were studied *in vivo*, as resistance modifiers in human pancreatic cancer xenograft bearing mice.

In this work we showed that the combination of the SILA-421 MDR retardant and the paclitaxel mitotic inhibitor shows a synergistic interaction *in vivo*. After that we compared the results with the earlier experiments (with SILA-409). SILA-421 was more effective than SILA-409. For the *in vivo* tests we used immunosuppressed CBA mice with *s.c.* implanted pancreatic carcinomas. These animals were treated with a combination of SILA-421 (10 mg/kg) and Taxol (7 mg/kg b.w./twice a week) for 4 weeks. Every week, tumours were measured and the actual tumour volume was calculated. At the end of the 4th week the mice were sacrificed and the tumours were removed. The removed tumours were fixed in formalin and embedded in paraplast. Slides were created from every tumour and an immunohistochemical method was applied with Ki-67 and p170 antibodies. Digital photos were made from the slides after the rates of the Ki-67 positive cells were measured with an image analysing software (IMAN).

The continuous measure of the tumour volume brought an interesting result: there was a tumour volume decrease by more than 50% on the 2nd week and this volume was stable until the end of the experiment.

SILA-421 has a better inhibiting potential than its predecessor, SILA-409, and the results show that SILA-421 has also an apoptotic effect, too. The results indicate that the SILA mdr inhibitor compounds are promising agents in the future cancer therapy.

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SITE-SPECIFIC MUTATION IN THE CATALYTIC DOMAIN OF TOPOISOMERASE IIβ ALTERS ENZYMATIC ACTIVITY AND DNA DAMAGE RESPONSE *IN VIVO*

Kenichi Chikamori, Adrian G. Grozav, Toshiyuki Kozuki, Michael Kinter, Belinda Willard, Dale R. Grabowski, Anni H. Andersen*, Ram Ganapathi and Mahrukh K. Ganapathi

Cleveland Clinic Foundation, Cleveland, Ohio, USA; *Aarhus University, Aarhus, Denmark

The DNA topoisomerase (topo) II enzyme catalyzes topological transformations of DNA and regulates DNA metabolic events, such as DNA replication and recombination, chromosome condensation and segregation and transcription. Two related topo II isozymes, topo IIα and topo IIβ, are present in mammals. Although both enzymes catalyze similar enzymatic reactions *in vitro*, they exhibit differential patterns of expression and discrete physiologic functions *in vivo*, suggesting that these isozymes are distinctly regulated. Since, both enzymes are phosphorylated and phosphorylation, of at least the topo IIα isozyme, regulates enzyme activity and function, in this study we initiated mass spectrometry experiments to identify relevant phosphorylation sites in topo IIβ. During the course of these studies, we serendipitously identified an essential tyrosine (Y661), located in the catalytic domain, which when mutated to phenylalanine (F) significantly reduced the decatenation activity of topo IIβ and reduced topo II-DNA cleavable complex formation *in vitro*. Topo IIβ-deficient Jurkat or topoIIα-depleted HTETOP cells expressing Y661F mutant protein were less sensitive to topo IIβ-targeted drugs as compared to cells expressing wild-type (WT) protein. HTETOP or topo II-depleted BJ201 yeast cells expressing Y661F topo IIβ were also less sensitive to ionizing radiation than those expressing WT protein. Although Y661, along with three other sites, serine (S) 1400, threonine (T) 1431 and S1550, was initially identified by mass spectrometry as a potential phosphorylation site (+80 Da modification), the modification at Y661 was subsequently determined to be due to oxidative bromination (+80 Da) which occurred during cleavage of topo IIβ with cyanogen bromide and trypsin. Nevertheless, this reactive site, which plays a critical role in topo IIβ function, was the only site found to influence topo IIβ activity. Interestingly, mutation of the equivalent tyrosine, Y640, in topo IIα, minimally (~2-fold less) influenced the decatenation activity of this topo IIα and did not alter drug sensitivity to topo II-targeted drugs. These data suggest that topo IIβ is regulated by a novel mechanism, involving oxidation at Y661. This mechanism may be important for modulating biological functions that respond to DNA damage and oxidative stress, wherein an oxidative environment is present.

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NOVEL PREDICTOR GENES IN OVARIAN CARCINOMA

Luis Rojas-Espaillat, Amanda Nickles-Fader, Nabila Rasool, Susan A.J. Vaziri, Toshiyuki Kozuki, Dale Grabowski, Charles Biscotti, Richard Drake, Chad Michener, Peter Rose, Jerome Belinson, Mahrukh K. Ganapathi and Ram Ganapathi

Cleveland Clinic Foundation, Cleveland, Ohio, USA

The high incidence and mortality of ovarian cancer coupled with the difficulty in detecting the disease in the early-stages poses a major clinical challenge. Among the 75-80% of the patients who present with stage III or stage IV ovarian cancer at the time of diagnosis, 70-80% of the women respond to the standard treatment protocol, which involves surgery followed by 6-8 courses of chemotherapy with platinum, *e.g.* carboplatin, and a taxane, *e.g.* paclitaxel. However, ~30% of patients that exhibit disease progression either during treatment or within 6 months after the last course of chemotherapy, also do poorly with second-line treatment. This remains a major problem in the management of ovarian cancer

since no markers predicting early relapse or genes functionally linked to chemo-sensitivity to platinum and/or taxane therapy have been validated.

Our gene expression profiling studies of stage III ovarian or peritoneal carcinoma using cDNA microarray technology have identified a set of three genes that are independent predictors of response to chemotherapy and clinical outcome. Two genes, cisplatin resistance-associated over-expressed protein (*CROP*) and proteosome subunit alpha type 3 (*PSMA3*) are significantly up-regulated in ovarian tumors of patients who have undergone primary surgical cytoreduction and who respond poorly to first-line therapy, whereas the third gene, chemokine C-C motif ligand 2 (*CCL2*), is significantly down-regulated in this same patient cohort.

In ovarian cancer cell lines the A(-2518)G polymorphism in the promoter region of the CCL2 gene, which has been previously reported to affect expression of the *CCL2* gene, led to reduced expression of *CCL2* mRNA and protein. Testing the function of *CCL2* in ovarian cancer cell lines demonstrated that reduced expression was correlated with resistance to cisplatin and paclitaxel. Ectopic overexpression of CCL2 in an ovarian cancer cell line led to enhanced sensitivity to paclitaxel but not cisplatin and also reduced tumor cell invasion in a Biocoat® Matrigel invasion assay. The overexpression of CROP and PSMA3 was also correlated with resistance to cisplatin in several cell culture models of ovarian cancer. Cisplatin treatment in a dose-dependent manner led to a 2- to 3-fold increase in accumulation of cells in G_2+M phase (indicative of enhanced DNA damage) in *CROP* siRNA-transfected cells in which *CROP* mRNA was down-regulated >70% compared to similar cells transfected with a scrambled siRNA.

In the long-term, establishing the functional role of *CCL2, CROP* or *PSMA3* as predictors of response to chemotherapy and outcome in patients with advanced stage ovarian cancer will allow for the development of novel strategies for detection and treatment.

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ROLE OF CASEIN KINASE I ISOZYMES δ AND ε IN REGULATING ENZYME ACTIVITY OF TOPOISOMERASE IIα

Adrian G. Grozav, Kenichi Chikamori, Toshiyuki Kozuki, Dale R. Grabowski, Michael Kinter, Anni H. Andersen*, Mahrukh K. Ganapathi and Ram Ganapathi

Cleveland Clinic Foundation, Cleveland, Ohio, USA; *Aarhus University, Aarhus, Denmark

Human topoisomerase (topo) IIα is an essential enzyme that regulates DNA topology. In human leukemia HL-60, cells resistance to topoisomerase (topo) II targeting drugs, such as etoposide, was found to be associated with site-specific hypophosphorylation of topo IIα. Resistance to etoposide was found to be calcium dependent, since resistance to etoposide could be mimicked in sensitive cells treated with the intracellular Ca^{2+} chelator, 1,2-*bis*(2-aminophenoxy)ethane-*N,NN',N'*-tetraacetic acid (BAPTA-AM). We subsequently identified serine-1106 (Ser-1106) in the catalytic domain of topo IIα as a major phosphorylation site, phosphorylation of which regulated enzymatic activity of topo IIα. Ser-1106 was found to be hypophosphorylated in sensitive cells treated with BAPTA-AM or in etoposide-resistant cells. The observed correlation of Ser-1106 hypophosphorylation with etoposide resistance in cell culture model systems was confirmed in blast cells from patients with acute myelogenous leukemia, wherein hypophosphorylation of Ser-1106 was correlated with reduced etoposide-induced apoptosis.

Since Ser-1106 lies within the consensus sequence for the acidotrophic kinases, casein kinase (CK) I and CKII we tested the functional role of these enzymes to phosphorylate this site *in vivo* using HL-60 and colon cancer HCT-116 cells. The CKI inhibitors, CKI-7 and IC-261, reduced phosphorylation of Ser-1106 and decreased formation of etoposide-stabilized topo II-DNA cleavable complex. In contrast, the CKII inhibitor, 5,6-dichlorobenzimidazole riboside (DRB), did not affect formation of etoposide-stabilized topo II-DNA cleavable complex. Since, IC261 specifically targets the Ca^{2+}-regulated isozymes, CKIδ and CKIε, we examined the effect of down-regulating these enzymes on Ser-1106 phosphorylation. Down-regulation of these isozymes with targeted si-RNAs led to hypophosphorylation of the Ser-1106-containing peptide. However, si-RNA-mediated down-regulation of CKIIα and CKIIα' did not alter Ser-1106 phosphorylation. Further, reduced phosphorylation of Ser-1106, observed in HRR25 (CKIδ/ε homologous gene)-deleted *S. cerevisiae* cells transformed with human topo IIα, was enhanced following re-expression of human CKIε. Down-regulation of CKIδ and CKIε also led to significantly reduced formation of etoposide-stabilized topo II-DNA cleavable complex.

These results provide strong support for an essential role of CKIδ/ε in phosphorylating Ser-1106 in human topo IIα and in regulating enzyme function.

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ROLE OF NONCODING RNAS IN TUMORIGENESIS

Alan Garen[1] and Xu Song[2]

[1]Yale University School of Medicine, 333 Cedar St., New Haven, CT 06520, USA;
[2]Sichuan University, 29 Wangjiang Road, Chengdu, Sichuan 610064, P.R. China

We present a model for generating tumor cells from differentiated cells and stem cells, based on a mechanism of gene regulation involving the tumor-suppressor protein PSF

and a PSF-binding noncoding RNA (ncRNA). PSF contains a DNA-binding domain (DBD) that binds to the promoter of a gene and represses transcription, and two RNA-binding domains (RBDs) that bind a ncRNA, releasing PSF and activating transcription. The model has six postulates. (i) Proliferation of stem cells and tumor cells is driven by multiple proto-oncogenes, some of which which are repressed by PSF and activated by PSF-binding RNAs. (ii) The level of PSF-binding RNAs is high in stem cells and tumor cells, preventing repression of proto-oncogenes by PSF and promoting proliferation. (iii) PSF-binding RNAs disappear in most stem cells at the end of embryogensis, enabling PSF to repress proto-oncogenes and initiate differentiation. (iv) Somatic mutations in a stem cell or differentiated cell can result in a high level of PSF-binding RNAs, generating a clone of tumor cells. (vi) The level of PSF-binding RNAs is reduced by miRNAs that degrade PSF-binding RNAs, and is increased by *ras* oncogenes that induce synthesis of PSF-binding RNAs. Thus, the model predicts that PSF and miRNAs that degrade PSF-binding RNAs act as tumor-suppressors, and PSF-binding RNAs and *ras* oncogenes act as tumor-promoters. The evidence for the model will be discussed.

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NEW APPROACHES IN COLORECTAL CANCER GENETICS

Maria Gazouli

Department of Biology, School of Medicine, University of Athens, Michalakopoulou 176, 11527 Athens, Greece

Colorectal cancer (CRC) is the third most common cancer and fourth-leading cause of cancer death worldwide. Lifetime risk in Western European and North American populations is around 5%. Both genetic and environmental factors contribute to disease etiology, with about one-third of disease variance attributed to inherited genetic factors. For complex diseases, such as CRC, genetics research in human populations, remarkable progress has been made in recent times with the publication of a number of genome-wide association scans (GWAS) and subsequent statistical replications. These studies have identified new genes and pathways implicated in disease, many of which were not known before. Until very recently, the defined genetic contribution to CRC comprised rare, high-penetrance variants in a few genes (DNA mismatch repair genes, *APC, SMAD4, BMPR1A* and *MUTYH*). In a genome-wide association study to identify loci associated with CRC, 555,510 single nucleotide polymorphisms were genotyped in different populations including 747 Greek CRC cases and 850 controls. The GWAS studies revealed that common genetic variations in the 8q23.3, 8q24, 10p14, 11p23, 15p13, and 18q21 (*SMAD7*) regions also contribute to CRC risk. As well as providing risk estimates for population groups, identification of CRC risk loci provides new insights into disease causation. Studies of the mechanisms by which these genetic associations impact CRC risk could lead to the development of small molecule interventions for chemoprevention and chemotherapy.

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CYTOTOXIC AND ANTIPROLIFERATIVE ACTIVITIES OF COBALT (II) COMPLEXES WITH DIFFERENT LIGANDS ON CULTURED TUMOR CELLS

R. Alexandrova[1], P. Genova[2], E. Gardeva[1], R. Toshkova[1], M. Kirilova[3], G. Miloshev[3], M. Lalia-Kantouri[4], L. Patron[5] and O. Costisor[6]

[1]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Science, Acad. G. Bonchev Str., Bl., 1113 Sofia;
[2]National Institute of Infectious and Parasitic Diseases, 44A Stoletov Blvd, 1233 Sofia;
[3]Institute of Molecular Biology, BAS, Acad. G. Bonchev Str., Block 21, 1113, Bulgaria;
[4]Aristotle University of Thessaloniki, Chemistry Department, Laboratory of Inorganic Chemistry P.O.Box 135, Thessaloniki 54124, Greece;
[5]Institute of Physical Chemistry "I.G.Murgulescu", Splaiul Independentei 202, sect. 6, 060021 Bucharest, Romania;
[6]Institute of Chemistry Timisoara of the Romanian Academy, 24 Mihai Viteazu Blvd, RO-300223, Timisoara, Romania

Cobalt is one of the most important trace elements in the world of animals and humans. Various cobalt containing compounds have been reported to express antineoplastic properties. The aim of this study was to evaluate the influence of 19 newly synthesized cobalt (II) complexes with different ligands (aminoacids, cholic acids, Mannich bases, mixed ligands) on cultured tumor cells. The following model systems were used in the experiments: 1) Permament cell lines from human (carcinoma of the larynx Hep-2, rhabdomyosarcoma RD64, glioblastoma multiforme 8 MG BA, breast cancer MCF-7 and erythroleukemia K562), rat (transplantable sarcoma induced by Rous sarcoma virus strain Schmidt-Ruppin), murine (myeloma P3U1) and chicken (transplantable hepatoma induced by the myelocytomatosis virus Mc29) origin; 2) primary cultures from myeloid tumor in hamster induced by Graffi mouse leukemia virus. The influence of the compounds on cell viability and proliferation as well as cytopathological changes were studied by MTT test, neutral red uptake cytotoxicity assay, colony-forming method, autoradiography, neutral and alkaline variants of single cell gel electrophoresis and acridine orange staining. The results obtained revealed that the compounds tested express time- and concentration-dependent cytotoxic and antiproliferative

activities. The compounds with the most promising antitumor properties were found among the complexes with mixed ligands 2-hydroxy-benzophenones and nitrogenous bases enR. Some of them were found to reduce cell viability by 50% (IC_{50}) when applied at concentrations <5 μg/ml for 24 h.
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IN VIVO EFFECT OF ASSORTED CHEMOPREVENTIVE MOLECULES ON DMBA-INDUCED ONCO-SUPPRESOR GENE EXPRESSION IN CBA/CA MICE

Péter Gergely[1], Géza Mezey[2], László Őrfi[3] and Istvan Ember[4]

[1]Institute of Forensic Medicine, University of Debrecen, H-4012, Debrecen, Nagyerdei krt. 98.;
[2]Department of Neurosurgery, University of Debrecen, H-4012, Debrecen, Nagyerdei krt. 98.;
[3]Institute of Pharmaceutical Chemistry, Semmelweis University of Medicine, H-1092 Budapest, Hőgyes E. Street 7;
[4]Institute of Public Health, University of Pecs, Szigeti str 12, H-7624 Pecs, Hungary

We studied six molecules purported to be antineoplastic and chemoprevenitve protein tyrosine kinase (PTK) inhibitors. These molecules are plant-derived flavonoids. In order to determine whether promising antineoplastic activity would extend to anticarcinogenic properties, the effects of these molecules on the DMBA (7,12- dimethylbenz[α]anthracene)-induced expression of H-ras and p53 in isolated RNA from liver, lung, kidney, thymus and bone marrow of CBA/Ca inbred mice was investigated. Elevated expression of oncogenes after treatment with DMBA has been reported previously.

Administration of these molecules simultaneously with DMBA, 24 h prior to or 24 h after the DMBA treatment characteristically modified the DMBA-induced expression of the genes in the twenty-four hour "short-term" experiments, showing the chemoprevenitve and/or antineoplastic effect of the observed agents. Coadministration of DMBA and agent 72, a styrylacrylonitrile compound, resulted in a significant decrease of the DMBA induced expression of H-ras in all organs; p53 expression decreased in liver, lung and bone marrow, increased in kidney and thymus. Administration of agent 72 24 h after the DMBA treatment reduced the DMBA-induced H-ras expression in all examined organs. The p53 expression decreased in all organs except the kidney. Administration of agent 72 24 h prior to the DMBA treatment reduced the DMBA-induced expression of H-ras in all organs. The expression of p53 induced by DMBA was elevated in kidney, in the bone marrow and lung, but decreased in thymus. Agent 72 alone decreased the expression of H-ras in all organs. The p53 expression increased significantly in liver and kidney but decreased in the lung and remained almost the same in the thymus and in the bone marrow. As previously described, this method is capable of screening promising anti-neoplastic molecules.

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NOVEL ROLE OF NEURONAL CALCIUM SENSOR-1 (NCS-1) AS PROGNOSTIC FACTOR AND THERAPEUTIC TARGET IN BREAST CANCER

S. Germano[1], M. Clynes[1], S. Kennedy[2], J.P. Mehta[1], E. Kenny[1], E. Ryan[1], P. Doolan[1], P. Gammell[1], H. Joyce[1], M. Perez de Villarreall[1], A. Hill[2], B. O'Daly[2], R. Linehan1, D. Cronin[1], C. Daly[1], S. Glynn[1] and L. O'Driscoll[1]

[1]National Institute for Cellular Biotechnology, Dublin City University, Dublin 9;
[2]St. Vincent's University Hospital, Dublin 4, Ireland

Neuronal calcium sensor-1 (NCS-1), formerly known as Frequenin, is a calmodulin-related EF-hand Ca^{2+} binding protein expressed mainly, but not uniquely, in neurons and neuroendocrine cells. NCS-1 modulates synaptic transmission and plasticity and has recently been identified as a regulator of neurite outgrowth and neuronal survival. Notwithstanding the diverse and well characterised biological functions of this protein in neurons, its involvement in cancer has never been studied. Here, the functional and clinical relevance of NCS-1 in breast cancer was investigated. In a microarray analysis of 103 invasive breast tumours and non-cancerous breast biopsies, we identified NCS-1 to be expressed in a sub-group of tumours (approximately 12% of cases), but to be undetectable in normal breast tissue. Multivariate and univariate analyses revealed NCS-1 expression to be associated with estrogen receptor (ER)-negativity and shortened times to relapse (p=0.0421) and death (p=0.0032) from time of diagnosis. To investigate the possible role of NCS-1 in breast tumour cell biology, we characterised the biological effects of this gene *in vitro* using breast cancer cell lines. To achieve this, NCS-1 expression was analysed in a panel of breast cancer cell lines using quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) and, following initial screening, two highly invasive triple negative (ER-, PR-, HER-2-) cell lines, Hs578T and HCC1143, as well as the weakly invasive MCF7 cell line, were selected for gain of function experiments. NCS-1 cDNA was introduced into these cells and associated effects on cell proliferation, motility and invasiveness were evaluated, compared to empty-vector transfected control cells. While monolayer cell growth rate

was not affected, anchorage-independent growth was significant enhanced by NCS-1, with a remarkable tendency for NCS-1 transfected cells to form larger colonies compared to the control cells. Motility of NCS-1 transfected cells was also significantly enhanced. Interestingly, the invasive ability, measured by monitoring invasion through matrigel in a Boyden Chamber assay, was also markedly increased. Our results suggest that NCS-1 may contribute to the malignant phenotype of breast cancer cells and may represent a new prognostic biomarker and potential therapeutic target for a sub-group of breast cancer patients.

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SURGERY IN BYZANTIUM

S. Geroulanos

Onassis Cardiac Surgery Center, Athens, Greece

Surgery in Byzantium with respect to the research and results achieved in many other medical fields remained more or less impenetrable, despite its major contribution in conservation, development and transfer of ancient knowledge. There are descriptions of ordinary or even of severe operations, thus making their study a fascinating subject. At least three of the most important physicians/authors of Byzantium, like Oreibassios, Aetios from Amida and Pavlos Aeginitis described and performed amazing operations. A selection of them is listed below:

General Surgery: Strumectomy (with reference to the importance of recurrent nerve), herniotomy and herniorrhaphy, hydro- and varicocele operation, entero- and omphalocele, laparocentesis, gastrorrhaphy, lymph node and ganglion excision, hexadactily operation. Abscess incision and drainage, liver and "spleen" abscess drainage, scrofulosis, panaritium incision, ingrowing-nail operation, removal of foreign bodies, finger-, arm- and leg-amputation.

Neurosurgery: Trepanation, craniotomy, elevation of impressed bone segments, several operations on cranial fractures.

Angiology: Ligation of arteries, arteriotomy, arterial resection in temporal arteriitis, aneurysmectomy, varicectomy (various methods including stripping), haemostasis by compression, ligation, cauterization and haemostyptics.

Ophthalmology: Blepharotomy, blepharoplasty of distichiasis, lagophthalmus operation, ektropion operation, anabronchismus, hydatic cyst removal, chalazion, pterygium, catarrhact operations, eyelid-sty treatment.

Ear-Nose-Throat: Rhinoplasty after nose operation, resection of nose-polyps, retroauricular acoustic pore opening, plastic ear-reconstruction, reposition of mandibula luxation, sialolithiasis operation, uvulectomy, tonsillectomy, tracheostomy.

Breast surgery: Tumorectomy, mastectomy, combined mamma-preserving tumor resection and cauterization, incision of breast abscess, removal of milk-stones, excision of necrosis and fistulas, gynaecomasty operation.

Thoracic surgery: Rib resection, drainage of empyema, separation of siamese twins (the first in the world).

Gynaecology: Transvaginal hysterectomy, drainage of uterus empyema, operation of cervix varices, nymphectomy, operation of hymenal, vaginal and uterus atresia, resection of pudendal, vaginal and cervical condylomas.

Obstetrics: Major improvements in irregular birth, use of forceps, embryotomy, support of genitals during birth, manual extraction of placentar rests, manual cleansing of uterus in postpartal infection, abortion.

Urology: Circumcision and phimosis operation, hypospadias operation (building neo-urethra), resection and cauterization of condylomas, catheterization of bladder and lavage, transurethral and transvaginal cystolithotrypsie, transvaginal and transperineal cystolithiasis removal, castration, hermaphrodites operation.

Proctology: Haemorhoidectomy (incl. ligation and cauterization), fistulotomy and fistulectomy, seton technique, perianal abscess drainage, anal atresia operation.

Traumatology: All possible reductions of simple and complicated bone fractures and repositions of luxation, extractions of arrows, spears *etc*. including gastro-, duodeno-, jejuno-, colono-, and vesico-rrhaphy.

The importance of the Byzantine texts in surgery were recognized in France, where they became by special decree obligatory texts for the medical students of the Sorbonne from the beginning of the 17th century till the end of the 19th century.

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IMMUNOMODULATION OF CANCER CELL MIGRATION AND INVASION USING RECOMBINANT *CAMELID* NANOBODIES AGAINST CYTOSKELETAL PROTEINS

Veerle Delanote[1,2], Anske Van den Abbeele[1,2], Sarah De Clercq[1,2], Ariane De Ganck[1,2], Katrien Van Impe[1,2], Veerle De Corte[1,2], Berlinda Vanloo[1,2], Ciska Boucherie[1,2], Joël Vandekerckhove[1,2] and Jan Gettemans[1,2,3]

[1]Department of Medical Protein Research, VIB, B-9000 Ghent;
[2]Department of Biochemistry, Ghent University, Faculty of Medicine and Health Sciences, Albert Baertsoenkaai 3, B-9000 Ghent, Belgium

Invasion and metastasis of cancer cells relies on dynamic reorganization of the actin cytoskeleton which is the driving force for cellular motility. Actin-associated or actin-binding proteins aid in this process by virtue of their ability to interact

reversibly with actin and actin filaments. The expression level of quite a few actin binding proteins is up-regulated in cancer cells, and many studies have demonstrated a correlation between the expression of selected actin-binding proteins and cancer cell motility and/or invasion *in vitro* and *in vivo*.

Instead of modulating the expression level of actin-associated proteins, we developed small recombinant antibodies (so-called nanobodies) raised against cell motility factors and use these as intrabodies. Obviously these antibodies do not affect the expression of their target antigen but inhibit biological functions. Nanobodies (~15 kDa) against gelsolin and other actin-associated proteins were raised in llamas or alpacas which have the unique property of expressing heavy chain antibodies that are devoid of light chains. The antigen-binding region of these antibodies (VHH) can be cloned, thus raising the possibility of developing small protein inhibitors against structural intracellular polypeptides. In addition, nanobodies can be used for tracing their target in living cells, thereby circumventing protein overexpression. They are also suitable for immunoprecipitation and Western blotting.

We show that selected nanobodies bind their target with nanomolar affinity. Moreover, epitope mapping demonstrates their selective interaction with conformational epitopes (*i.e.* calcium-induced conformational changes in proteins). Examples are discussed showing inhibition of biochemical activities of L-plastin, an actin-bundling protein, by nanobodies. Moreover, this was associated with inhibition of filopodia formation in PC-3 cells.

Importantly, expression of nanobodies against L-plastin or gelsolin in cancer cells reduced motility and *in vitro* invasion of these cells to the same extent as siRNA. This approach allows one to study the role of (actin-associated) proteins in tumorigenesis at the level of the protein. Nanobodies could be further developed into bona fide therapeutic molecules.

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G-QUADRUPLEX STRUCTURES IN ANTICANCER AND ANTIVIRAL THERAPY

C. Giancola

Department of Chemistry "P. Corradini", University of Naples Federico II, Via Cintia 80126, Naples, Italy

Guanine-rich nucleic acid sequences can adopt quadruplex structures (G-quadruplexes) stabilized by quartet layers of Hoogsteen paired residues (1). G-quadruplexes are further stabilized by monovalent cations as sodium and potassium. G-rich sequences are found at the ends of chromosomes as telomeric protein complexes, and in a number of biologically significant regions of the genome, such as gene promoter regions and sequences associated with human diseases. The biomedical relevance of quadruplexes is essentially due to two potential applications: in anticancer therapy and in the design of novel aptameric nucleic acids. The first application comes from the observation that G-quadruplexes formation at the 3'-end of telomeres may inhibit the telomerase enzyme, an event which can induce cancer cells to escape senescence (2, 3). The second application is due to the ability of aptamers based on the quadruplex motif to specifically bind selected proteins (4). A specific quadruplex has been found to bind and inhibit α-thrombin, an important protein with multiple function in homeostasis, and some quadruplexes have resulted to be potent antiviral inhibitors against HIV. Many structural and biophysical methods are devoted to study native quadruplexes and their interactions with both proteins and small molecules (5). The energetic aspects of both quadruplex assembly and quadruplex-ligand interactions are discussed here. Recent studies on physico-chemical properties and antiviral activity of quadruplex structures obtained from synthetic aptamers are also discussed.

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ANGIOGENESIS AND MATRIX METTALLOPROTEINASES: THE COMMON PATHWAY TO CANCER PROGRESSION AND THEIR IMPACT ON PANCREATIC DUCTAL AND AMPULLARY CARCINOMA

George Giannopoulos, Kitty Pavlakis, Aikaterini Parasi, Nikolaos Kavatzas, Dina Tiniakos, Antigoni Karakosta, Nikolaos Tzanakis and George Peros

4th Surgical Department, Attikon Hospital, University of Athens, Athens, Greece, Apostoli 2 st, 185 37 Piraeus, Greece

Aim: To investigate the expression of metalloproteinase (MMP)-2, MMP-9 and tissue inhibitor of MMP (TIMP)-2 in pancreatic ductal and ampullary carcinoma and to test the findings for correlation with angiogenesis and several clinicopathological parameters. *Materials and Methods:* Paraffin sections from 32 pancreatic ductal adenocarcinomas and 17 ampullary carcinomas were assessed for the expression of MMP-2, MMP-9 and TIMP-2, by immunohistochemistry. Stromal and epithelial staining were evaluated separately. Moreover, sections stained immunohistochemically with anti-CD34 antibody were evaluated by image analysis for the

quantification of microvessel density (MVD). *Results:* In pancreatic ductal adenocarcinoma, lower levels of glandular TIMP-2 were found in poorly differentiated tumors, while high glandular TIMP-2 expression was significantly associated with better survival. The age of the patients and the degree of differentiation of the tumor were identified as independent prognostic parameters. No relation was found between the expression of MMPs, TIMP or angiogenesis and the parameters under consideration. In ampullary adenocarcinoma, strong expression of glandular MMP-2 was associated with higher MVD values. Moreover, lymph vessel invasion was associated with higher stromal TIMP-2. *Conclusion:* In pancreatic ductal adenocarcinoma, TIMP-2 may have a more crucial role in prognosis than MMP-2, MMP-9 or angiogenesis. In ampullary adenocarcinoma, MMP-2 expression correlated with MVD, supporting its postulated role in angiogenesis.

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MULTI-TARGETING OF U87 GLIOMA CELLS BY SUNITINIB AND LAPATINIB

Efstathia Giannopoulou, Andreas A. Argyriou, Angelos K. Koutras, Fotinos Dimitrakopoulos and Haralabos P. Kalofonos

Clinical Oncology Laboratory, Division of Oncology, Department of Medicine, University Hospital of Patras, Patras Medical School, Greece

Aim: Sunitinib and Lapatinib are currently used for the treatment of solid tumors. The efficacy of these agents is not widely tested neither in experimental models nor in clinical trials of malignant gliomas and therefore we studied the effect of these agents, applied either alone or combined, on U87 glioma cells. *Methods:* Sunitinib and lapatinib were applied, either separately or in combination, in the U87 cells at doses of 10 nM, 100 nM and 1 μM. To determine whether these agents affect the proliferation of U87 glioma cell lines, the 3-[4,5-dimethylthiazol-2-yl]-2,5 dimethyltetrazolium bromide assay was used. Apoptosis was detected using annexin V/propidium iodide detection assay, migration assay was performed in 24-well microchemotaxis chambers and MMP-2 levels were measured by zymography. *Results:* Both agents, administered either alone or combined, decreased cell proliferation in a dose-dependent manner 48h after their application. The inhibition of agent's combination was statistically different than the inhibition of each agent alone. We also found that apoptosis was increased and invasion of U87 cells was inhibited either by each agent alone or their combination. Finally, the application of both agents did not result in alteration of MMP-2 levels. *Conclusion:* Our results showed that the application of sunitinib and/or lapatinib appears to exhibit effects on proliferation, apoptosis and invasion of U87 cell line. When applied alone, sunitinib appears to be a more potent inhibitor than lapatinib.

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DIFFERENTIAL EXPRESSION OF METALLOPROTEINASE BETWEEN NORMAL MUCOSA OF PATIENTS WITH SPORADIC COLORECTAL CANCER AND OF NORMAL SUBJECTS: MAY PLAY A ROLE FOR NEOPLASTIC TRANSFORMATION?

Francesco Franceschi, Lucia Fini, Giovanni Gigante, Maria Assunta Zocco, Alberto Manno, Davide Roccarina, Bianca Giupponi, Guido De Marco, Alessandro Verbo, Claudio Mattana, Nicolò Gentiloni-Silveri, Claudio Coco, Giovanni Gasbarrini and Antonio Gasbarrini

Internal Medicine, Department of Surgical Sciences, Catholic University of Rome, Italy

The matrix metalloproteinase (MMP) family of enzymes represents a group of about 20 proteins which have been involved in promoting of human malignancies, including colorectal cancer (CRC). We hypothesized that a genetic impairment of MMP and/or their inhibitors in the normal colonic mucosa may create a favorable environment for neoplastic transformation. Therefore, we designed a study aimed at assessing the gene expression profile of MMP and their inhibitors in samples of sporadic CRC tissue, normal colonic mucosa of patients with sporadic CRC and normal mucosa of healthy subjects. *Methods:* Ten patients with sporadic CRC and 10 healthy sex and age matched subjects were enrolled. Samples of CRC, normal mucosa of patients with CRC and normal mucosa of healthy subjects were collected. Total RNA was extracted from all samples; cRNA was hybridized with the human U133A array set (22.000 genes), which also include 20 MMP genes and their inhibitors. Gene expression profile was compared among groups. The expression level of MMP and their inhibitors found to be increased or decreased in gene chip analyses was further studied by real-time PCR to validate microarray results. *Results:* CRC tissue *vs*. normal mucosa of CRC patients: overall, a differential expression in 7 MMP genes has been observed by both microarray analysis and RT-PCR. In particular, the gene expression of MMP-1, MMP-2, MMP-7, MMP-8, MMP-9, MMP-12, and MMP-20 was found to be increased in CRC specimens compared to the normal mucosa of the same patients. Concerning TIMPs, only TIMP-1 was increased in CRC tissues. Normal mucosa of CRC patients *vs*. normal mucosa of healthy subjects: overall, 6 MMP genes were differently expressed between groups by both microarray analysis and RT-PCR. In particular, MMP-11, MMP-12, MMP-15 and MMP-20 were up-regulated in the normal mucosa of CRC patients, while MMP-14 and MMP-19 were

down-regulated in the same samples. Concerning TIMPs, TIMP-3 was decreased in the normal mucosa of CRC patients. *Conclusion:* Several MMP genes are differentially expressed in CRC tissue, normal mucosa of patients with sporadic CRC compared to healthy subjects. Interestingly, some of those genes, such as MMP-11 and MMP-12, which were up-regulated in the normal mucosa of CRC patients, have also been described to play a role in CRC, while MMP-15 and MMP-20 have been reported to be associated to other kind of cancers. Furthermore, MMP-19, a protein involved in colonic epithelial cell proliferation, and TIMP-3, which were down-regulated in the normal mucosa of patients with CRC, are known to decrease during malignant transformation. Based on these findings, we hypothesize that the pattern of expression of those genes seen in the normal mucosa of patients with sporadic CRC may create a favorable environment for neoplastic transformation and progression.

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ANTIMUTAGENIC AND ANTICANCER EFFECTS OF BLACK TEA POLYPHENOLS THEAFLAVINS AND THEARUBIGINS IN MULTIPLE TEST SYSTEMS

Udayan Bhattacharya, Babli Halder, Rashmi Roy, Sibabrata Mukhopadhyay and Ashok K. Giri

Molecular and Human Genetics Division, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Jadavpur, Kolkata- 700 032, India

Tea is a highly consumed and popular beverage throughout the world. During fermentation tea catechins are polymerized by polyphenols oxidase to form theaflavins (TF) and thearubigins (TR). TF and TR are the most exclusive polyphenols of black tea that account for 3-6% and 12-18% of dry weight of black tea. We have observed significant antimutagenic effects of TF and TR in multiple test systems. A statistically significant inhibition of chromosomal aberration and micronuclei formation was found *in vitro* in human lymphocyte cultures. Tea polyphenols exert their potent anticancer activity and appear to be the ideal agents for chemoprevention. Even though few previous reports show the anticancer effects of TF through apoptosis, nevertheless the potential effect of TR has not been appraised. This study investigated the induction of apoptosis in human skin cancer cells after treatment of TR and TF. We report that both TF and TR could exert inhibition of human A431 (epidermoid carcinoma) and A375 (malignant melanoma) proliferation without adversely affecting NHEK (normal human epidermal keratinocytes) cells. Growth inhibition of A375 cells occurred through apoptosis, as evident from cell cycle arrest at G_0/G_1 phase, increase in early apoptotic cells, externalization of phosphatidyl serine and DNA fragmentation. In our pursuit to dissect the molecular mechanism of TF and TR induced apoptosis in A375 cells, we investigated whether cancer cell death is being mediated by mitochondria. In our system, Bax translocation to mitochondria persuaded depolarization of mitochondrial membrane potential, facilitated ROS (reactive oxygen species) generation, cytochrome *c* release in cytosol and induced activation of caspase -9, caspase -3 as well as PARP (poly (ADP-ribose) polymerase) cleavage. Our intricate investigations on apoptosis, also explained that, TF and TR augmented Bax/Bcl2 ratio and upregulated the expression of p53 and p21. The TF and TR also inhibited phosphorylation of the cell survival protein Akt. Furthermore, we have found that TF and TR can also upregulate phosphorylated JNK and phosphorylated p38 in A375 cells. These observations raise speculation that TF as well as TR might exert chemopreventive effect through cell cycle arrest and induction of apoptogenic signals via mitochondrial death cascade and stress activated MAPKinase pathways may also be involved in inducing apoptosis in human skin cancer cells.

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CHLOROQUIN CONVERTS MOLECULAR IODINE-INDUCED AUTOPHAGY IN MDA-MB-231 TO APOPTOTIC CELL DEATH: IMPLICATIONS FOR OVERCOMING CHEMOTHERAPEUTIC RESISTANCE

M.M. Godbole[2], Preeti Singh2, Geeta Rao[2], Vishwamohan, Sanjay Annarao[1], Raja Roy[1], Kalyan Mitra[3] and V.K. Bajpai[3]

[1]Department of Endocrinology, Centre of Biomedical Magnetic Resonance,
[2]Sanjay Gandhi Postgraduate Institute of Medical Sciences,
[3]Electron Microscopy Facility, Central Drug Research Institute, Lucknow, India

Autophagic mechanism is known to provide survival advantage and chemoresistance in various types of cancer. Molecular iodine (I_2) has been shown to cause apoptotic cell death independent of estrogen receptor and p53 status in breast cancer cell lines (MCF-7, MDA-MB-231, MDA-MB-453, ZR-75-1, T-47D). In MCF-7 cells, apoptotic cell death has been shown to be independent of caspase activity and is mediated through AIF. Non-evident apoptosis in MDA-MB-231 in response to I_2 treatment led us to investigate its cell death mechanism in detail. Electron microscopic observations and immunofluorescence performed in molecular iodine-treated MDA-MB-231 cells showed an increase in acidic vacuoles and autophagosome formation, and subsequent autolysosome formation confirmed the autophagy. Blocking of various stages of I_2-induced autophagy by PI3 kinase inhibitors (LY294002 and 3-MA), bafilomycin (lysosomal fusion inhibitor) resulted in enhanced cell death, indicating that autophagy provides survival advantage to MDA-MB-231 cells. The evidence of

autophagy is further supported by enhanced expression of Beclin, reduced Bcl-2, and increased LC-3 cleavage seen on Western blot.

Recent evidence that the anti-malarial drug chloroquin inhibits chemotherapeutic regimen-induced autophagy leading to increased cell death in CNS lymphoma is promising. To know whether the chloroquin-mediated increased therapeutic efficacy is drug/system-specific or is a more generalized phenomenon, we investigated its effect in I_2-treated MDA-MB-231 cells. Co-treatment with I_2 and chloroquin resulted in increased cytotoxicity, accumulation of sub-G1 cell fraction, nuclear fragmentation with enhanced caspase-9 activation and reduced pro-caspase-3, indicating that chloroquin redirects the cells undergoing autophagy under the influence of molecular iodine to dominant apoptotic mechanism of cell death. The evidence provided so far indicates that the chloroquin probably increases the therapeutic efficacy of the drug regimen in a ubiquitous manner. This switch over ability from autophagy to apoptosis provided by chloroquin makes it a potential adjuvant to overcome the survival advantage conferred by molecular iodine on MDA-MB-231 cells.

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A COMPLEX KARYOTYPE IS AN INDEPENDENT PROGNOSTIC FACTOR FOR CHILDREN WITH MDS – DATA FROM THE EUROPEAN WORKING GROUP OF MDS IN CHILDHOOD (EWOG-MDS)

Gudrun Gohring[1], Kyra Michalova[2], Berna Beverloo[3], David Betts[4], Jochen Harbott[5], Oskar Haas[6], Gitte Kerndrup[7], Laura Sainati[8], Eva Bergstraesser[4], Henrik Hasle[9], Jan Starý[10], Monika Trebo[6], Marry van den Heuvel-Eibrink[3], Marco Zecca[11], Alexandra Fischer[12], Peter Noellke[12], Franco Locatelli[11], Brigitte Schlegelberger[1] and Charlotte M. Niemeyer[12]

[1]Institute of Cell and Molecular Pathology, Medical School Hannover, Germany;
[2]Center of Tumor Cytogenetics, Charles University, General Faculty Hospital, Prague, Czech Republic;
[3]Department of Clinical Genetics and Sophia Children's Hospital, Erasmus University, Rotterdam, the Netherlands;
[4]Department of Oncology, University Children's Hospital, Zuerich, Switzerland;
[5]Department of Pediatric Medizine, University, Giessen, Germany;
[6]St. Anna Children's Hospital, Vienna, Austria;
[7]Institute of Pathology, University Hospital, Odense, Denmark;
[8]Clinica Oncoematologica Pediatrica, University of Padova, Italy;
[9]Department of Pediatrics, Aarhus University Hospital Skejby, Aarhus, Denmark;
[10]Deptartment of Pediatric Hematology and Oncology, University Hospital Motol, Prague, Czech Republic;
[11]IRCCS Policlinico San Matteo,Department of Pediatrics, University of Pavia, Italy;
[12]Department for Pediatric of Adolescent Medicine, University Hospital, Freiburg, Germany

To study the clinical significance of recurrent chromosome aberrations in childhood MDS, cytogenetic data of 394 consecutive children with refractory cytopenia (RC) (N=215), RAEB (N=141) and RAEB-T (N=38) analyzed in the regional cytogenetic reference centers and registered in the prospective study EWOG-MDS 98 between 1998 and 2005 were evaluated. At diagnosis, a karyotype could be defined in 279/394 patients (pts) (71%). No karyotype was obtained in 16% of pts with RC as compared to 8% pts with RAEB and RAEB-t ($p<0.001$). Clonal chromosome aberrations were more common in pts with advanced MDS (RAEB and RAEB-T, 61%) compared to RC (29%), and in pts with secondary (69%) compared to primary MDS (36%) ($p<0.001$). Monosomy 7 was the most frequent aberration occurring with similar frequency in RC (47% of abnormal karyotypes) as compared to advanced MDS (49%) and in primary (53%) compared to secondary (41%) MDS. In addition, aberrations typical for *de novo* AML, such as aberrations involving 11q23 or 3q, t(6;9) and del(9q), were noted in morphologically and clinically unequivocal MDS cases. Recurrent aberrations of adult MDS such as isolated del(5q), del(20q) and -Y were very uncommon, indicating a different pathogenesis of these cases. In pts with advanced MDS, there was no significant difference in overall survival (OS) of pts with normal karyotype (44%±18) as compared to pts with monosomy 7 (58%±19) and patients with other karyotypes (61%±22). However, pts with advanced MDS and a complex karyotype (defined by ≥3 chromosome aberrations, presence of structural aberrations and excluding clonal evolution of monosomy 7) had a lower OS (16% ±15, $p<0.01$). OS and event-free survival after hematopoietic stem cell transplantation (HSCT) in pts with complex karyotypes was inferior compared to that of pts with other cytogenetic aberrations ($p=0.012$ and 0.039, respectively). Within the group of pts with secondary MDS, complex karyotypes were found in MDS evolving from inherited bone marrow failure disorders or after radiochemotherapy, but absent in familial MDS and cases evolving from acquired aplastic anemia. As shown in a multivariate Cox analysis, advanced MDS, secondary MDS, the presence of a complex karyotype and HSCT were identified as independent prognostic factors for OS. Thus, this study demonstrates the prognostic significance of cytogenetic findings in advanced childhood MDS independent of HSCT.

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THE EFFECT *IN VITRO* AND *IN VIVO* OF HUMAN GALECTIN-8 VARIANTS; A POSSIBLE NEW THERAPY FOR INFLAMMATORY AND CANCER DISEASES

Itshak Golan[1,2] and Ira Golan[1,2]

[1]Institute of Life Sciences, Swansea University, Swansea, Wales;
[2]Kennedy Institute of Rheumatology, Imperial College, London, UK

In our recent publication (Eshkar-Sebban *et al*., J Immunol *179*: 1225-1235, 2007) we described a study shows by affinity chromatography, surface plasmon resonance and flow cytometry, that galectin-8 is a novel ligand of CD44. We further demonstrated that synovial fluid cells from rheumatoid arthritis patients (RA) contain new variants of human galectin-8 proteins and cell surface CD44. Both CD44 and galectin-8 proteins, possibly released from the inflammatory cells, were detected also in the joint fluid of the patients. We further revealed that at least part of galectin-8 and soluble CD44 form complexes in the RA synovial fluid that reduce the ability of the lectin to induce apoptosis in the inflammatory joint cells.

Hence, the RA model not only confirmed the receptor-ligand relationships between CD44 and galectin-8, but also unveiled the biological significance of this interaction in the regulation of the inflammatory cascade.

Knowing that the interaction between galectin-8 and integrins is mediated by the sugar moieties of the adhesion receptors and that this interaction can subsequently lead to apoptotic signaling, we predicted that galectin-8 can also be ligated to CD44. This assumption was based on the fact that, like integrins, CD44 is a glycosylated cell surface receptor that can deliver death signals. Challenging this prediction experimentally, we not only confirmed the receptor- ligand relationship between CD44 and galectin-8, but also showed that this interaction reduced the anti-inflammatory activity of the lectin.

In the present study, we are reporting our recent findings on new pro apoptotic agents, human galectin-8 variants, which can provide a novel diagnostic, prognostic and therapeutic approach for joint chronic inflammatory diseases and cancer.

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EFFECTS OF ISOFLURANE ON NFKB1, GADD45A JNK1 EXPRESSIONS IN THE VITAL ORGANS OF CBA/CA MICE

Balázs Kádár[1], Katalin Gombos[2], Eszter Szele[2], Gyula Gőbel[3], István Szanyi[3] and István Ember[2]

[1]Anaesthesiology and Intensive care Unit BAZ County Teaching Hospital, Miskolc;
[2]University of Pécs, Faculty of Medicine, Institute of Public Health, Pécs;
[3]Department of Otolaryngology Head and Neck Surgery, Faculty of Medicine, University of Pécs, Hungary

Background: Isoflurane is one of the most widely used volatile anaesthetics. It is administered to patients during general anaesthesia with the aim of minimizing the effect of external stimuli, such as surgical invasions. It was also found to be beneficial to patients undergoing operation with coronary heart disease by reducing myocardial stunning and infarct size. On the other hand isoflurane has also been reported to have genotoxic effects in humans, male Sprague–Dawley rats and in cell lines exposed to isoflurane. This genotoxic effect manifested as an increase in DNA single strand breaks by alkaline comet assay. In our present study we evaluated the expression of GADD45α, JNK1 (MAPK8) and NFKB1. GADD45α is a gene that is induced by DNA damage and strongly linked to the c-jun-N terminal kinase cascade and to the NFKB pathway, both playing important role in the regulation of apoptosis and cell survival. *Materials and Methods:* 5-week-old male CBA/CA mice were exposed to 2% isoflurane anaesthesia for 1 hour. Control animals were exposed to 90% Oxygen. Lungs, liver and kidneys of the animals were removed 3 and 6 hours after isoflurane exposure. Gene expressions were measured by quantitative real-time PCR on total RNA from the organs. For internal control we used HPRT. *Results:* Significant expression alterations of the three genes were seen 3 hours after the isoflurane exposure in the lungs and the kidneys. GADD45α and JNK1 showed paralell correlation in changes of expression, while NFKB1 had inverse activation compared to the other two genes. *Conclusion:* By inducing DNA damage and activating GADD45α, isoflurane exposure evokes the activation of JNK1 and NFKB1 through which can have an effect on the cell death program.

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ANALYSIS OF THE CONNECTIONS BETWEEN SIGNAL TRANSDUCTION MECHANISMS IN EARLY-STAGE THYROID TUMOURS

Katalin Gombos[1], Eszter Szele[1], László Puskás[2], László Kozma[3], Ferenc Juhász[4], Gyula Gőbel[5], István Szanyi[5] and István Ember[1]

[1]Institute of Public Health, Faculty of Medicine, University of Pécs, Szigeti út 12. H-7624 Pécs;
[2]Functional Genomical Laboratory, Biological Research Center of Hungarian Academy of Sciences, Szeged;
[3]Hematology Center of Kenézy County Hospital, Debrecen;
[4]1st Department of Surgery, University of Debrecen, Faculty of Medicine, Debrecen;
[5]Department of Otolaryngology Head and Neck Surgery, Faculty of Medicine, University of Pécs, Pécs, Hungary

Introduction: Microarray analysis offers the opportunity of screening transcriptional expression profile of neoplastic cells at the genomic level. Analysis and comparison of these molecular snapshots makes possible to identify characteristic steps of deregulation and dedifferentiation in tumorigenesis. This genomewide screening can reflect a momentary picture of global network of all transcriptional events in tumours providing wide insight into the connections of the signalling mechanisms driven in tumour cells. *Patients and Methods:* cDNA microarray with 20.000 human gene specific oligonucleotide was used to analyze benignant and early-stage malignant thyroid tumours of epithelial origin: follicular adenoma (n=8), follicular carcinoma (n=7) and papillary carcinoma (n=10) compared to normal thyroid tissue (n=20). *Results:* We compared microarray patterns of early-stage thyroid tumours looking particularly not for significantly modulated candidate genes, but a set of genes acting on similar antiapoptotic and signalling pathway, and the ones showing overlaps between the early-stage epithelial thyroid tumour types. We identified significant expression differences of 258 genes- underexpression of 233 and overexpression of 25 genes and focused on the overlapping genes between the different histological types. Among these genes we found a limited set acting on similar transcriptional pathways: through NF-κB and PPARγ pathway. Conclusion: The role of overlapping genes in histologically different tumours has not been clarified, but might represent early or pivotal steps of carcinogenesis. All investigated histiotypes of tumours contained significantly modulated genes acting on NF-κB regulatory pathway. Our findings suggest that modulation of NF-κB signalling plays crucial role in early thyroid carcinogenesis.

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FEEDING PURIFIED GLYCEROL FROM BIODIESEL TO CBA/CA MICE: EFFECTS ON SINGLE STRAND DNA DAMAGE INDUCIBLE GADD45A AND NFKB EXPRESSIONS

Eszter Szele[1], Katalin Gombos[1], András Kovács[2] and István Ember[1]

[1]University of Pécs, Faculty of Medicine, Institute of Public Health, Pécs;
[2]KUKK, K+F Kft., Budapest, Hungary

Background: In the European Union the turn towards renewable energy sources has increased the production of biodiesel from rapseed oil (rapseed oil methyl ester) leaving glycerol as a valuable by-product. Glycerol is a natural liquid substance registered in the European Union as a feed additive E422. Glycerol could become attractive for ruminants if the amount of the by-product exceeds the capacities of the pharmaceutical and chemical industries to process glycerol. In the present situation glycerol purification is costy and it is necessary to evaluate the final glycerol by-product of different techniques and levels of purification. In our study we aimed to evaluate a promising, neutralized, filtered and distilled glycerol with acceptably high purity and with a methanol content lower than 0.1% called SZME2. We investigated the effect of this glycerol product on the expressions of DNA damage inducible genes GADD45α and NFKB, in case it was added to standard diet of CBA/CA mice, sensitive to carcinogen exposure. *Materials and Methods:* 5-week-old CBA/CA mice were administered SZME2 in the standard chaw pellet with a glycerol concentration of 10% of diet dry matter. Animals were given SZME2 fortified diet and tap water ad libitum for 3, 6 and 24 hours. Control animals consumed the standard chaw pellet and tap water. Liver, spleen and bone-marrow of the animals were removed during autopsy. Quantitative real-time PCR was carried out on isolated total RNA. Gene expression alterations of GADD45α and NFKB were calculated in HPRT percentage. *Results:* No significant changes were seen in gene expressions of GADD45α and NFKB in the liver and spleen at the three timepoints of administration of SZME2 in the diet. However a consequent and significant down-regulation of the two genes was observed in the bone marrow in both gender. *Conclusion:* Based upon our data we can not declare that methanol content under 0.1% of purified glycerol byproducts of biodiesel production have no effect on the homeostatic regulation at molecular level. Our investigation underline the necessity of animal carcinogenicity bioassay on large laboratory animal population before introducing purified glycerol byproducts on the market.

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A PERSONALIZED APPROACH TO DETERMINE PROGNOSTIC AND PREDICTIVE INDICATORS IN BREAST CANCER WITH GENE EXPRESSION PROFILING MICROARRAYS

Y. Gong[1], K. Yan[1], F. Lin[1], K. Anderson[1], C. Sotiriou[2], F. Andre[3], F.A. Holmes[4], V. Valero[1], D. Booser[1], J.E. Pippen[5], S. Vukelja[6], H. Gomez[7], J. Mejia[1], L.J. Barajas[8], K.R. Hess[1], N. Sneige[1], G.N. Hortobagyi[1], L. Pusztai[1] and W.F. Symmans[1]

[1]The University of Texas M.D. Anderson Cancer Center, Houston TX, USA;
[2]Institut Jules Bordet, Brussels, Belgium;
[3]Institut Gustave Roussy, Villejuif, France;
[4]US Oncology-Texas Oncology, Houston, TX;
[5]US Oncology-Texas Oncology, Sammons Cancer, Dallas, TX;
[6]US Oncology-Texas Oncology, Tyler Cancer Center, Tyler, TX, USA;
[7]Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru;

[8]Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico

Background: Despite a considerable decline in the mortality from breast cancer following systemic therapy, the biology of breast cancer remains poorly understood. This is because that the routinely-used clinicopathologic variables fail to fully capture the biologic heterogeneity. Gene expression microarrays may provide more sophisticate information than conventional biomarkers in predicting disease outcome and response to a specific systemic therapy on an individual basis. However, whether ER and HER2 status, two important biomakers, can be reliably measured from the comprehensive microarray data is unclear. *Methods:* we used gene expression data of 495 breast carcinomas to assess the correlation between ER and HER-2 mRNA levels and clinical status of these genes (as determined by immunohistochemical and/or fluorescence *in situ* hybridization). Data from 195 fine-needle aspiration (FNA) samples was used to define mRNA cutoff values and the accuracy of these cutoffs was assessed in two independent data sets: 123 FNA samples and 177 tissue specimens (ie, resected or core-needle biopsied tissues). Profiling was conducted at two institutions using the same platform (the Affymetrix U133A GeneChip). All data were uniformly normalised using dCHIP software. *Results:* ER and HER-2 mRNA levels correlated closely with routine receptor status measurements in all three data sets. Spearman's correlation coefficients ranged from 0.62 to 0.77. The defined ER mRNA cutoff identified ER-positive status with the overall accuracy of 88-96%; and the defined HER-2 mRNA cutoff identified HER-2-positive status with the overall accuracy of 89-93%. *Conclusion:* ER and HER2 gene expression can be reliably measured from the comprehensive microarray data. Integration of ER and HER2 mRNA expression with multigene signatures from the same microarray data may refine and improve their predictive power. These findings may represent an important step towards personalized treatment.

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XRCC3 GENE POLYMORPHISM IN BLADDER CANCER

Kamil Fehmi Narter[1], Arzu Ergen[1], Bedia Agachan[1], Umit Zeybek[1], Uzay Gormus[1,2] and Turgay Isbir[1]

[1]Istanbul University, Institute for Experimental Medicine, Department of Molecular Medicine, İstanbul;
[2]Istanbul Bilim University, Faculty of Medicine, Department of Biochemistry, İstanbul, Turkey

Smoking is known to be one of the most important factors that increases the risk of bladder cancer and it is thought to damage DNA *via* free oxygen radicals. In this case, the repair capacity of the DNA is important to protect from cancer. XRCC3 has roles in double-strand breaks repair of DNA, and plays a role in maintaining chromosome stability. We aimed to investigate *XRCC3* gene polymorphism that cause Thr241Met change in bladder cancer. There were 55 bladder cancer patients and 39 control cases in our study. The polymorphism analysis was performed by PCR-RFLP. It was found that there was a significant difference in carrying T allele of *XRCC3* gene between patient and control groups. We found that the T allele has 4.87 times protective capacity against bladder cancer risk. Detailed investigations using larger study groups, may allow the prognosis of patients to be determined.

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SERUM MYELOPEROXIDASE LEVEL AND GENE POLYMORPHISM IN ENDOMETRIUM CANCER

Sibel Bulgurcuoglu Kuran[1], Arzu Ergen[1], Uzay Gormus[1,2], Bedia Agachan[1], Umit Zeybek[1] and Turgay Isbir[1]

[1]İstanbul University, Institute for Experimental Medicine, Department of Molecular Medicine, İstanbul;
[2]İstanbul Bilim University, Faculty of Medicine, Department of Biochemistry, İstanbul, Turkey

Endometrial cancer is one of the most frequent types of cancer of the female genital system. Increasing age, obesity, hypertension and diabetes mellitus are known to be risk factors in creating this cancer. It is also thought that free radicals can activate inflamatory responses and with the contribution of hypoxia, they can cause DNA damage. Myeloperoxidase (MPO) is found in neutrophils and monocytes and catalyses reactions to produce hypochlorous acid, which is toxic to bacteria and is also a long-lived oxidant that can lead to activation of some procarcinogens causing damage to DNA. We aimed to investigate the levels of MPO activity in endometrial cancer patients while also determining whether the *MPO* gene polymorphism can increase the tendency to create endometrial cancer or not. There were 39 endometrial cancer patients and 39 control cases. Serum MPO levels were measured by enzyme-linked immunosorbent assay (ELISA) and *MPO* gene -463G/A polymorphisms were determined by PCR-RFLP. MPO levels were not significantly different between control and patient groups. A allele carriers were significantly more frequent in the patient group than in the control group (p=0.037) but no difference existed in G allele carriers. MPO levels were higher in the AA genotype patient group than other genotypes, but there was no such significance in the control group. Serum MPO levels were higher in the homozygote AA genotype group. As MPO is an enzyme that causes production of hypochlorous acid, our results show that this activity increment in the AA genotype might contribute to the increased tendency for endometrial cancer.

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MESENCHYMAL STEM CELLS DERIVED FROM NON-SMALL CELL LUNG CANCER AND NORMAL LUNG TISSUE DIFFER IN THEIR GENETIC STABILITY, GENE EXPRESSION PROFILES, AND FUNCTIONAL BEHAVIOR

Sandra Gottschling[1], Anna Jauch[2], Martin Granzow[2], Ruprecht Kuner[3], Thomas Muley[1], Elizabeth Chang Xu[1], Felix F.J. Herth[1], Hans Hoffmann[1], Hendrik Dienemann[1], Anthony D. Ho[4], Michael Thomas[1] and Michael Meister[1]

[1]Clinic for Thoracic Diseases GmbH at University Hospital Heidelberg, Amalienstr. 5, 69126 Heidelberg;
[2]Insitute for Human Genetics, University Heidelberg, Im Neuenheimer Feld 366, 69120 Heidelberg;
[3]Division of Molecular Genome Analysis, German Cancer Research Center, Im Neuenheimer Feld 280, Heidelberg;
[4]Department Medicine V, University Hospital Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

Background: The stromal microenvironment plays a vital role in induction and maintencance of solid tumors (1). Various studies in prostate, breast, and ovarian cancer showed genetic and functional alterations in the peritumoral stroma. However, corresponding data in lung cancer are lacking (2). Here we provide a systematic and comparative analysis of genetic and functional properties of autologous non-small cell lung cancer (NSCLC) and normal lung tissue (NLT) stromal cells with special respect to mesenchymal stem cells (MSC). *Methods:* Stromal cells derived from NSCLC specimens and microscopically normal lung tissue of newly diagnosed lung cancer patients were isolated and propagated in culture. Cells were analyzed for their mesenchymal character by flow cytometry and their osteogenic and adipogenic differentiation potential. Genetic analyses were performed by multicolor FISH. Gene expression was analyzed by Affymetrix HG U133 Plus 2.0-based arrays. The most differentially expressed genes were confirmed by real-time PCR. Moreover, cells were analyzed for growth kinetics and colony-forming capacity. Chemosensitivity towards cisplatin was analyzed by annexin V/propidium iodide, trypan blue stain, and colony forming assays. *Results:* Compared to NLT, cell preparations derived from NSCLC specimens were four-fold enriched in fibroblast colony-forming units (CFU-*f*). In pure NSCLC-MSC cultures, about twice as many CFU-*f* (5.5±1.2% *vs*. 2.8±0.8%) were present than in NLT-MSC cultures. CFU-*f* in NLT-MSC cultures declined significantly from passage 8 on whereas CFU-*f* yield in NSCLC-MSC cultures remained stable over several passages. This was in line with faster growth kinetics and an up to 27-fold *vs*. 12-fold expansion of NSCLC-MSC after 10 passages. NSCLC-MSC displayed a significantly reduced cisplatin sensitivity, with a delayed onset of apoptosis and a higher survival of CFU-*f*. M-FISH analyses of 5 paired MSC preparations at passage 5 demonstrated polyploidy and unbalanced translocations in NSCLC-MSC whereas NLT-MSC showed either no genetic alterations or at best balanced translocations, indicating a higher genetic stability. In line with the functional differences, NSCLC-MSC showed a higher expression of genes such as *ENDOD1* (DNA repair), *ANGPT-1* (angiogenesis), *MTP18* (antiapoptotic), and *CD109* (primitive MSC marker) and a lower expression of genes such as *QSOX1* (fibroblast quiescence), *SEMA3C* (MSC ageing), or *C6orf32* (myogenic differentiation). *Conclusion:* NSCLC specimens are enriched in genetically unstable primitive mesenchymal cells with increased proliferative capacity and reduced chemosensitivity to cisplatin. These cells might modulate and sustain the cancerogenic process and affect the response to chemotherapy.

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2 Hill R, Song Y, Cardiff RD *et al*: Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. Cell *123*: 1001-1011, 2005. Tuhkanen H, Anttila M, Kosma V-M *et al*: Genetic alterations in the peritumoral stromal cells of malignant and borderline epithelial ovarian tumors as indicated by allelic imbalance on chromosome 3p. Int J Cancer *109*: 247-252, 2004.

ENDOD1: endonuclease domain containing 1, ANGPT-1: angiopoietin-1, MTP18: mitochondrial protein 18 kDa, QSOX1: quiescin Q6 sulfhydryl oxidase 1, SEMA3C: sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin), C6orf32: chromosome 6 open reading frame 32, 3C

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MOLECULAR PATHOLOGY OF LOBULAR BREAST CANCER

Anna Goussia

Department of Pathology, Medicine School, University of Ioannina, Ioannina 45110, Greece

Lobular breast cancer, *in situ* (LCIS) and invasive (ILC), is a distinct subset of tumors, based on morphology, genetics and biology. Morphologically, LCIS and ILC are characterized by a proliferation of uniform, loosely cohesive cells with mild nuclear atypia. Cytogenetic studies have shown that LCIS and ILC have relatively low numbers of changes compared with ductal breast cancer. Moreover, the molecular genetic profiles of LCIS and ILC are similar, suggesting the common clonality of the lesions and the precursor role of LCIS.

The main features of lobular malignancies are gain of 1q, loss of 16q and loss or down-regulation of E-cadherin. Loss or down-regulation of E-cadherin occurs by a combination of loss of heterozygosity, gene mutation or promoter silencing

leading to inactivation of the gene and it is manifested in routine histology practice by immunonegativity for E-cadherin. The E-cadherin negativity has been used as a diagnostic feature of lobular carcinomas. Since some ductal carcinomas may also be E-cadherin negative, either totally or partially, E-cadherin immunonegativity must be interpreted with caution, taking into account the overall morphological context. Loss of E-cadherin function is thought to contribute to the histological appearance of lobular breast cancer, i.e. lack of cohesive architecture with single cells invading through the stroma, ability of metastatic spread to serosal cavities. Other distinctive but not unique molecular features of lobular breast cancer are EGFR1, HER2/neu and cyclin D1 negativity or absence/rarity of amplification of the above genes as well as ER and PR positivity.

Recently, a pleomorphic variant of lobular carcinoma (PLC) has been described. In pleomorphic LCIS and ILC, neoplastic cells display several of the histological and molecular features associated with classic lobular carcinomas but exhibit more conspicuous nuclear atypia and pleomorphism and in addition, they frequently show apocrine differentiation. Although molecular data are limited, it is apparent up to now that PLC have overlapping genetic characteristics with those of classic lobular carcinomas (1q+/16q-/11q-, E-cadherin negative, E-cadherin mutation) and those of high grade ductal carcinomas [p53 stabilization, HER2/neu overexpression, gain of 8q, gain of 17q24-q25, loss of 13q and amplification of 8q24 (MYC), 12q14 (MDM2), 17q12 (HER2/TOPO2A) and 20q13 (ZNF217)]. Therefore, PLC seem to evolve along a similar molecular pathway to the classic lobular carcinoma, but moreover, have a high grade phenotype through molecular changes associated with high grade ductal carcinoma. Molecular alterations found in PLC, that are typical of high grade tumors, are likely to drive the more aggressive biology of PLC.

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TARGETING THE EUKARYOTIC TRANSLATION INITIATION FACTOR 4E (EIF4E) FOR CANCER THERAPY

Jeremy R. Graff

Eli Lilly and Company, Indianapolis, IN, 46285, USA

Eukaryotic Translation Initiation Factor 4E (eIF4E) plays a pivotal role in cellular mRNA translation, binding the cap structure at the 5' end of cellular mRNAs and delivering these mRNAs to the eIF4F translation initiation complex. A substantial body of evidence has accumulated in the past 18 years implicating enhanced eIF4E activity in cellular transformation, tumorigenesis and metastatic progression. In human cancers, eIF4E expression is commonly elevated with disease progression in many tumor types including lymphomas as well as cancers of the head and neck, breast, colon, bladder and lung. We now show that eIF4E activation is universally and significantly increased in human and experimental prostate cancers. In human cancers, elevated eIF4E activation is significantly associated with reduced patient survival.

In experimental models, eIF4E overexpression can drive cellular transformation, tumorigenesis, invasiveness and metastases by selectively and disproportionately enhancing the translation of select mRNAs that code for the critical proteins that promote and sustain the phenotypes necessary for malignancy-uncontrolled growth (c-myc, cyclin D1), angiogenesis (VEGF), survival (BCL-2, survivin), and invasion (MMP-9). In addition, eIF4E overexpression facilitates autocrine growth and survival *via* activation of both the ras and AKT signaling pathways. Reduction of eIF4E expression in highly metastatic, ras-transformed experimental cancer models effectively blocks tumor growth and invasiveness as well as spontaneous and experimental metastasis, suppressing the expression of MMP-9, CD44v6 and ODC and restoring expression of the metastasis suppressor nm-23. These data clearly implicate eIF4E as an attractive anticancer therapeutic target.

Exploiting advances in antisense oligonucleotide (ASO) chemistry, we have developed eIF4E-specific ASOs with the tissue stability and nuclease resistance necessary for systemic, anticancer therapy. These ASOs specifically target the eIF4E mRNA for RNAse-H mediated destruction, repressing expression of eIF4E and the eIF4E- regulated proteins VEGF, cyclin D1, survivin, c-myc, and Bcl-2. In multiple human cancer cell lines, the 4EASO robustly induces apoptosis independent of cell cycle phase and, in endothelial cells, directly blocks the formation of vessel-like structures. Most importantly, intravenous administration selectively and significantly reduces eIF4E expression in human tumor xenografts, significantly suppressing tumor growth. As in cultured cells, systemic 4EASO administration significantly induced apoptosis in xenograft tissue (8X vs. control) and significantly reduced the number of Ki-67 + cells within the xenograft tumors as well. Because these ASOs also target murine eIF4E, we assessed the impact of eIF4E reduction in normal tissues. Despite reducing eIF4E levels by 80% in mouse liver, eIF4E-ASO administration did not affect body weight, organ weight or liver transaminase levels. Collectively, these data therefore provide the first direct, *in vivo* evidence that tumor tissues would be more sensitive to the effects of eIF4E inhibition than normal tissues, a differential effect consistent with the conceptual understanding that eIF4E activity is elevated in, and required by, tumor tissue to sustain the expression of key growth and survival factors that contribute to malignancy. These data have now prompted eIF4E-ASO clinical trials for the treatment of human cancers.

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FOLLOW-UP STUDY OF PATIENTS WITH A NEW DIAGNOSIS OF GLIOBLASTOMA MULTIFORME TREATED WITH TEMOZOLOMIDE AND THALIDOMIDE

A. Gramaglia[1], E. Novelli[2], A. Ravasio[1], S. Nava[1], F. Mattana[1], M. Mapelli[1], C. Bassetti[1], V. Cerreta[1] and G.F. Baronzio[1]

1Department of Radiation Oncology and Medical Physics, Policlinico di Monza;
2Department of Biostatistics, Gruppo Policlinico di Monza, Italy

Objective: The chemotherapeutic agent temozolomide and the antiangiogenetic agent thalidomide have both demonstrated antitumor effects in patients with glioblastoma multiforme (GBM). The objective of the study was to determine if the combined strategy of temozolomide and thalidomide with radiotherapy is associated with an improved median survival. The efficacy and tolerability of temozolomide alone and in combination with thalidomide were explored in a single-institution 13 years experience. *Methods:* From April 1993 to May 2006, one hundred and seventy patients with GBM underwent microsurgical tumor extirpation and radioterapy: 82 (48.2%) patients received no additional treatment (C), in 42 (24.7%) patients temozolomide was given alone (T) and 46 (27.1%) patients had a combined chemotherapy of temozolomide and thalidomide (TT). Radiotherapy parameters were a dose of 45 Gy delivered in 2.5-3 Gy fractions over 3-4 weeks, followed by a boost dose of 20 Gy delivered in 4-5 Gy fractions in 1 week. Temozolamide was administered starting at the end of radiotherapy with a dose of 200 mg/m^2 daily for 5 days, every 4 weeks. Thalidomide was started at the end of radiotherapy with a dose of 20 mg and escalated by 20 mg every week depending on patient tolerance, to a maximum of 100 mg daily. *Results:* Median survival, starting from the first radiotherapy, was 54 weeks (95% CI, 50-58 weeks) in C-patients, 83 weeks (95% CI, 36-130 weeks) in T-patients and 86 weeks (95% CI, 65-106 weeks) in TT-patients. Compared to the C-patients, the median survival of those who received temozolomide alone or in combination with thalidomide was 86 weeks (95% CI, 70-102 weeks), with a significant improvement in survival outcome (log-rank=6.613, p=0.010) (Figure). Temozolamide and thalidomide were well tolerated and only mild to moderate toxicities were observed. *Conclusion:* The strategy of combining temozolomide, thalidomide and radiotherapy in the treatment of GBM appears to be well tolerated and with favourable survival outcome. The addition of thalidomide in association with temozolamide does not offer a significant advantage over temozolamide alone.

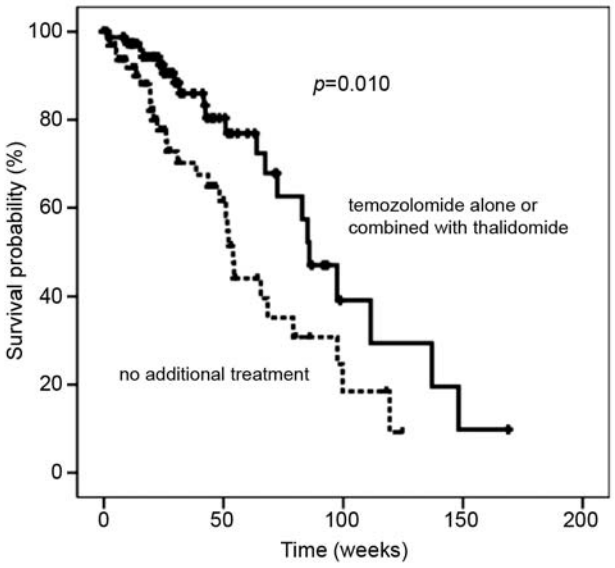

Figure. *Kaplan-Meier estimates of overall survival from time of first radiotherapy for patients with temozolomide alone or in combination with thalidomide (solid line) and patients with no additional treatment (dotted line).*

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INCREASED SURVIVAL IN GLIOBLASTOMAS AND ANAPLASTIC ASTROCYTOMAS TREATED WITH CONFORMAL RADIOTHERAPY AND HYPERTHERMIA

Gianfranco Baronzio[1], Vincenzo Cerreta[1], Chiara Bassetti[1], Marco Mapell[1], Alessandro Romorini[2] and Alberto Gramaglia[1]

[1]Radiotherapy and Hyperthermia Department, Policlinico di Monza, Via Amati 111, Monza;
[2]Neurology Unit, Magenta Hospital, Magenta (Mi), Italy

Introduction: Malignant gliomas and astrocytomas represent a class of aggressive neoplasms that are generally resistant to conventional therapies. The basic approach to treatment involves a combination of surgery, radiotherapy and chemotherapy. Among chemotherapeutic agents Nitrosoureas (CCNU) and Temozolomide (TMZ) have a certain activity against gliomas and astrocytomas. Recently, TMZ was demonstrated to be well tolerated and active as a single agent or in combination with radiotherapy. The median survival time for high grade gliomas is 10-12 months and the prognosis is dismal. New therapeutic approaches are justified. Several studies *in vitro* on glioblastomas have demonstrated that hyperthermia plus chemotherapy has a higher cytotoxicity than chemotherapy alone. Furthermore, heat has a cytotoxic effect by itself and an anti-vascular effect. This

last effect is of outmost importance due to the high neoangiogenesis present in these tumors. *Patients and Methods:* Between January 2001 and April 2008, 29 patients with aggressive brain tumors [11 glioblastomas (GBM), 14 astrocytomas (6 AstroIV, 8 Astro II degree, 1 Oligoastrocytoma), 2 ependymomas and 1 medulloblastoma] have been treated with conformal radiotherapy (CRT), chemotherapy and hyperthermia (HT). Twenty five of these patients (11 GBM and 14 astrocytoma) (9F, 16M; median age 44.6±9.85) have been treated with the CFRT + TMZ + HT and were eligible to be compared with a group of 27 patients with 18 GBM and 9 astrocytomas (12F, 15 M median age 50.93±13.9y) treated with CFRT and TMZ alone. All the patients of the two groups have been resected and later treated with CRT + TMZ or HT. HT was administered using a Synchrotherm radiofrequency (RF) device developed by DUER®, Vigevano, Italy. It consists of the following components: 1) a RF generator (13.56 MHz) 2) a pair of mobile plates or electrodes with independent superficial cooling system, 3) a heat exchanger, 4) a computerized control console. A thermal profiles to obtain a probable deposition of the energy were obtained by heating patterns produced in a static phantom under various conditions. The 25 patients were treated combining chemotherapy, radiotherapy and hyperthermia with the following sequence. HT was applied 2 h after CRT administration, and the patients used orally 120 mg of CCNU two hs prior HT or a median dose of 200 mg of temozolomide (TMZ). BCNU or TMZ was administered once per HT cycle, generally at the first application. A complete cycle of HT consisted of five applications, applied every 48 hs. Four mg of e.v. dexamethasone was started 1/2h before HT administered in the hypertonic solution of glucose 10% 500cc that lasted for all the treatment period (60'). *Results:* The group of patients treated with CFRT+TMZ+HT showed a significant increase in life survival ($p<0.001$) (Figure 1) and patients in follow-up of HT group were also over the median life survival *versus* 17.89±10.56 months of patients treated only with CRT + TMZ ($p<0.01$). 60% of the patients treated with CFRT+TMZ+HT were alive at 20 months. 36% of them had a life survival of more than 27 months. Comparing the entire group treated with HT *versus* CRT+TMZ the survival curve is even better (Figure 2). *Conclusion:* The life prolongation is clinically significant and these results suggest that effective HT may soon become a standard therapy associated to chemotherapy and radiotherapy for glioblastomas and astrocytomas. Notwithstanding these positive results we think necessary to increase the number of patients treated with hyperthermia, to produce a randomized study and to verify the possible side-effects of hyperthermia. Furthermore, we think useful to use a predictive method to verify the heat deposition since normally heat is not easily detected inside the brain like in other structures.

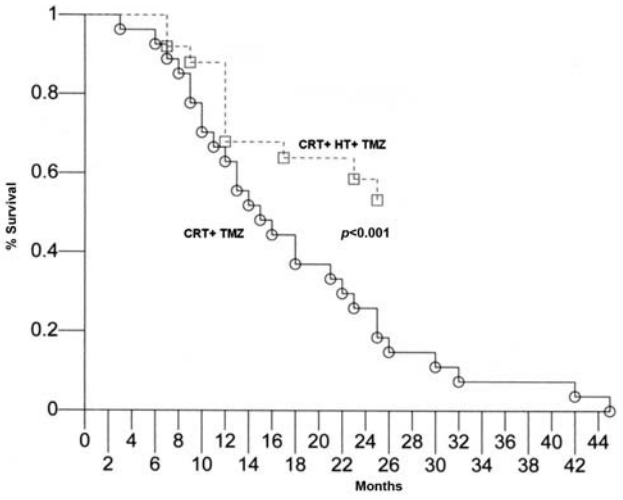

Figure 1. *Comparison of survival curves between patients treated with conformal radiotherapy plus CCNU/ TMZ (CRT+ CCNU/TMZ) and patients treated with CRT plus CCNU/ TMZ and capacitive hyperthermia (CRT+ CCNU/ TMZ + HT).*

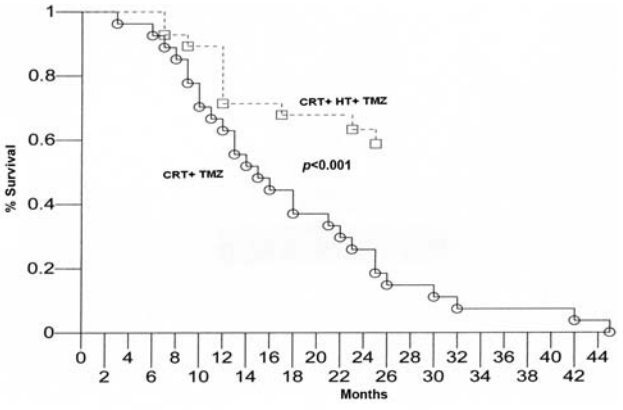

Figure 2. *Comparison of survival curves between patients treated with Conformal radiotherapy plus CCNU/ TMZ (CRT+ CCNU/TMZ) and all patients treated with CRT plus CCNU/ TMZ and capacitive hyperthermia (CRT+ CCNU/ TMZ + HT).*

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DIACYLGLYCEROL KINASE ALPHA IS REQUIRED FOR PROLIFERATION AND INVASION INDUCED BY GROWTH FACTORS AND CHEMOKINES

Paolo E. Porporato, Elena Rainero, Federica Chianale, Gabriella Ranaldo, Gema Tur Arlandis, Irene Locatelli, Gianluca Baldanzi, Nicoletta Filigheddu, Sara Traini, Miriam Gaggianesi and Andrea Graziani

Department of Clinical and Experimental Medicine, Università del Piemonte Orientale A.Avogadro, Novara, Italy

Diacylglycerol kinase enzymes (Dgks) convert diacylglycerol (DG) into phosphatidic acid (PA), thus acting as molecular switch between DG- and PA-mediated signalling. We previously showed that activation of Dgkα is required for growth factor-induced cell migration and proliferation through a mechanism involving formation of a complex with Src and (Cutrupi *et al*., EMBO J 2000; Baldanzi *et al*. Oncogene, 2004; Bachiocchi *et al*. Blood 2005; Baldanzi *et al*., Oncogene 2008). Moreover we showed that in epithelial cells, Dgkα is required for HGF-induced membrane ruffling, and regulates membrane targeting and activation of Rac (Chianale *et al*. Mol Biol Cell, 2007).

These data suggest that DGKα may play a role in the acquisition of an invasive phenotype. In order to set up an experimental system to investigate the role of DGKα in tumor progression *in vitro* and *in vivo*, we developed a lentiviral vector for shRNA-mediated constitutive knock down of DGKα. LV-shRNA-mediated down-regulation of DGKα in highly tumorigenic and metastatic MDA-MB-231 cells breast cancer cells results in the 60-70% reduction of serum-sustained cell proliferation, as well as inhibition of EGF-induced DNA synthesis. Expression of murine DGKα, resistant to human shRNA, rescues cell proliferation of MDA-MB-231, demonstrating that the proliferative defect is specifically dependent on DGKa expression.

In addition, shRNA-mediated knock-down of DGKα strongly impairs HGF- and SDF-1α- induced cell invasion in a 3D matrix and secretion of matrix gelatinases. The specificity of this effect is confirmed by the full rescue of invasive response to HGF and SDF-1α upon expression of shRNA-resistant murine DGKα. Furthermore, the signalling pathways by which DGKa regulates cancer cell invasive behaviour have been also investigated.

These data strongly suggest that DGKα plays a previously unrecognized pivotal role in tumor progression of breast carcinomas *in vitro*.

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ANTIPROLIFERATIVE EFFECT OF D-GLUCURONYL C5-EPIMERASE TO HUMAN BREAST CANCER

T. Eshchenko[1], N. Domanitskaya[1], T. Pavlova[2,3], V.I. Kashuba[2,4], G.I. Nepomnyashchikh[5], S.V. Aidagulova[3], E.R. Zabarovsky[2,3] and E.V. Grigorieva[1,2]

[1]Institute of Molecular Biology and Biophysics, SD RAMS, Novosibirsk, Russia;
[2]Karolinska Institute, MTC, Stockholm, Sweden;
[3]Institute of Molecular Biology, Moscow, Russia;
[4]Institute of Molecular Biology and Genetics, Kiev, Ukraine;
[5]Institute of Regional Pathology and Pathomorphology SD RAMS, Novosibirsk, Russia

Introduction: D-glucuronyl C5-epimerase (GLCE) is one of the key enzymes in biosynthesis of heparan sulfates – the polysaccharide part of heparan sulfate proteoglycans (HSPG) located on the cell surface and in the extracellular matrix. HSPGs play important roles in cell adhesion, differentiation, and growth and alterations in their structure/composition may have important consequences on tumour invasion and metastasis. Thus, GLCE could be involved in any processes where appropriate heparan sulfate structure is important. *Materials and Methods: D-glucuronyl C5-epimerase* expression in different human normal tissues and breast tumours was estimated by multiplex and qreal-time RT-PCR. For a functional study, *GLCE* was cloned into the vectors pETE/Bsd and pCEP4 for expression in mammalian cells. The effect of the restoration of *GLCE* expression on cell proliferation *in vitro* was investigated for breast cancer cell line MCF7 using CyQUANT NF Proliferation Assay. *Results:* It was shown that *GLCE* gene was expressed mainly in human breast and lung tissues, with less expression in thyroid, thymus and kidney. The significant down-regulation of epimerase expression was detected in human breast tumours. A restoration of D-glucuronyl C5-epimerase expression in the breast cancer cells suppressed their proliferation *in vitro*. *Conclusion:* The obtained results represent the first data about the possible antimitotic activity of *D-glycuronyl C5-epimerase* in breast cancer cells. A decrease of the epimerase expression in different types of cancer and its ability to suppress the proliferation of tumour cells reveal D-glycuronyl C5-epimerase as a potential new marker and target for cancer diagnosis and treatment.

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PROSPECTIVE COHORT COMPARISON OF FLAVONOID TREATMENT IN PATIENTS WITH RESECTED COLORECTAL CANCER TO PREVENT RECURRENCE

Harald Hoensch[1], Bertram Groh[2], Lutz Edler[3] and Wilhelm Kirch[2]

[1]Marienhospital Darmstadt, Martinspfad 72, Darmstadt D-64285;
[2]Department of Clinical Pharmacology, Medical Faculty, Technical University Dresden, Fiedlerstr. 27, Dresden D-01307;
[3]German Cancer Research Center, Division of Biostatistics, Im Neuenheimer Feld 280, Heidelberg D-69120, Germany

Aim: To investigate biological cancer prevention with flavonoids. The recurrence risk of neoplasia was studied in

patients with resected colorectal cancer and after adenoma polypectomy. *Methods:* Eighty-seven patients, 36 patients with resected colon cancer and 51 patients after polypectomy, were divided into 2 groups. One group was treated with a flavonoid mixture (daily standard dose 20 mg apigenin and 20 mg epigallocatechin-gallate, n=31) and compared with a matched control group (n=56) without flavonoid intervention. Both groups were observed for 3-4 years by surveillance colonoscopy and by questionnaires. *Results:* Of 87 patients enrolled in this study, 36 had resected colon cancer and 29 of these patients had surveillance colonoscopy. Among the flavonoid-treated patients with resected colon cancer (n=14), there was no cancer recurrence and only one tubular adenoma developed. In contrast, the cancer recurrence rate of the 15 matched untreated controls was 20% (3 out of 15), and adenomas (including 2 advanced adenomas) evolved in 4 of those patients (27%). The combined recurrence rate for neoplasia was 7% (1 out of 14) in the treated patients and 47% (7 out of 15) in the controls (p=0.027). Among the 51 polypectomized adenoma patients, 17 had surveillance colonoscopies (8 treated and 9 controls) and their adenoma recurrence rate was 50% in the treated and 30% in the control group. However, 2 incident adenomas with advanced histology were found in the untreated patients. *Conclusion:* Sustained long-term treatment with a flavonoid-mixture could reduce the recurrence rate of colon neoplasia in patients with resected colon cancer.

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SERUM LEPTIN LEVELS IN COLORECTAL CANCER PATIENTS

F. Guadagni[1], M. Roselli[2], A. Spila[1], R. D'Alessandro[1], I. Lucci[1], L. Polce[1], V. Formica[2], A. Laudisi[2], I. Portarena[2], R. Palmirotta[1] and P. Ferroni[1]

[1]Department of Laboratory Medicine and Advanced Biotechnologies, IRCCS San Raffaele Pisana, Rome; [2]Medical Oncology, Department of Internal Medicine, University of Rome "Tor Vergata", Rome, Italy

Background: Recent studies have indicated that some adipokines may significantly influence the growth and proliferation of tumor stroma and malignant cells within. Leptin, a product of the ob gene involved in the control of food intake and energy expenditure, may act as a potent mitogen and anti-apoptotic cytokine in colon cancer. Epidemiological studies have suggested that leptin is correlated with the risk of colorectal cancer (CRC) associated with obesity, the association being independent of body mass index (BMI), waist circumference and physical activity. Hence, increased leptin plasma levels were found in CRC patients. It is well known that tumor cells and/or tumor-associated leukocytes may produce inflammatory cytokines, in particular TNF-alpha. Circulating levels of this cytokine have been associated with the disease status of CRC patients. Recently, it has been demonstrated that TNF-alpha administration induced a prompt and dose-dependent increase in serum leptin levels. Thus, aim of this study was to evaluate the possible associations between leptin, TNF-alpha and clinicopathological variables of CRC patients at time of diagnosis of primary tumor. Moreover, a follow-up study was performed to analyze the possible prognostic value of pre-surgical leptin levels in patients with CRC. *Methods:* Baseline serum leptin (DBC Inc.) TNF-alpha (R&D Systems) and carcinoembryonic antigen (CEA, Abbott Labs.) levels were analyzed in 90 patients with histologically diagnosed primary (Stages A: 7, B: 34, C: 19 and D: 13, with a single resectable liver metastasis) or metastatic (liver: 8, peritoneum: 5, lung: 1 and multiple: 3) CRC treated at "Tor Vergata" Clinical Centre and followed for a median period of 3 years. As control group, in a 3:1 ratio, 30 control subjects (13 males, 17 females; mean age 59±12, ranging from 37 to 80 years) were also evaluated. The study was performed under the appropriate ethics approvals, and informed consent was obtained from each patient. *Results:* Serum leptin levels were higher in CRC patients [median (IQR): 8.8 (3.7-17.6) ng/ml] than control subjects [1.1 (0.3-3.8) ng/ml, p<0.0001]. Similarly, median TNF levels were higher in CRC patients [8.2 pg/ml] than control subjects [0.2 pg/ml, p<0.001]. Leptin directly correlated with TNF levels (Rho=49, p<0.001). Median leptin (10.9 ng/ml) levels of metastatic CRC were higher than those of primary CRC patients (7.7 ng/ml, p=0.034). Of interest, 47% of non metastatic CRC had leptin levels above the median compared with 71% of metastatic patients (p=0.07). Median follow-up of metastatic CRC patients was shorter (12.6 months) in patients with high leptin levels compared to those with normal levels (21.7 months, p=0.07). Cox proportional hazard regression model including age, sex, leptin, TNF and CEA levels showed that leptin was an independent predictor for overall survival in metastatic CRC (Cox-Mantel test 2.03, p=0.042). *Conclusion:* These results suggest that serum leptin levels might have a role in the biology of CRC and may be regarded as a useful prognostic indicator in patients with metastatic disease.

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PRL-3 EXPRESSION AND TUMOR BUDDING IN COLORECTAL CANCER

Katarzyna Guzińska-Ustymowicz

Departement of General Pathomorphology, ul.Waszyngtona 13, 15-889 Białystok, Poland

Background: Colorectal cancer is one of the most common types of cancer in Western countries including Poland. The

mortality from colorectal cancer is ranked as the second in Western countries, and third in Poland, amongst all types of cancer. Recent studies concerning the prognostic factors in colorectal cancer have paid attention to tumor budding as a potential prognostic factor. Morodomi *et al*. defined tumor budding as either bundles of five or more cancer cells occurring in a well-differentiated region (mainly the actively invasive area), showing tubular structures, which were classified as microtubular cancer nests, or isolated cancer cells without a distinct structure, which were classified as undifferentiated cells (1). Several published studies have indicated that tumor budding is associated with metastasis in colorectal cancers (2-4). PRL-3 is a newly discovered protein tyrosine phosphatase which would also degrade the extracellular matrix and was expressed in liver metastases derived from colorectal cancer. In this study, we investigated the relationship between tumor budding at the invasion front of colorectal cancer and expression of PRL-3 in the main mass of tumor, buds, lymph nodes and liver metastases. *Patients and Methods:* The samples were obtained from 49 selected patients with colorectal cancer, 20 adjacent normal epithelial (at least 5 cm distant from the tumor edge), 24 lymph node metastases, and 10 liver metastases were obtained from the Department of Pathomorphology, Medical University of Bialystok. Monoclonal antibody, Clone 3B6, against PRL-3 (Attogen Biomedical Research, USA) was used for immunohistochemistry. *Results:* Statistical analysis showed a correlation between tumor budding and lymph node metastasis. PRL-3 protein expression was observed in 1 out of 20 (5%) normal colorectal epithelia, 9 out of 49 (18.3%) primary colorectal cancer, 22 out of 24 (91.6%) lymph node metastasis and 9 out of 10 (90%) liver metastases, respectively. *Conclusion:* These results suggest that high PRL-3 expression may participate in the progression and metastasis of colorectal cancer.

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BEAMCATH® APPLICATION OR CONFORMAL TECHNIQUE IN RADIOTHERAPY IN EARLY-STAGE PROSTATE CANCER? BLADDER AND RECTUM TOXICITY

Solveig Hanssen

Department of Oncology, Section of Radiotherapy, University Hospital of North Norway, Tromsø, Norway

The aim of this study was to examine the toxicity of two external radiotherapy regimens employed in early-stage prostate cancer at the University Hospital of North Norway (UNN). During the last decade, the incidence of prostate cancer in Norway has been rising steadily, and a trend from surgery to radiotherapy in early-stage disease has been observed. In 1997, a new technique was developed in Sweden aiming to reduce the side-effects of radiotherapy. This technique utilises a special catheter, BeamCath®, to achieve a more accurate determination of the position of the prostate, and allow an increase in dosage. All ninety men who had undergone radiotherapy for early-stage prostate cancer at the Department of Oncology, UNN, in the time period February 2002 to March 2005, were included in a retrospective questionnaire based study. Eighty patients responded, and were divided into two treatment regimens. The "treatment group" (23 patients) had received 76 Gy with BeamCath® regimen and the "control group" (57 patients) had received 70 Gy employing a conformal technique. The patients were interviewed by telephone and six selected questions from validated questionnaires were used to clarify bladder, intestinal and sexual function. The BeamCath® technique was found to be associated with a lower median rectal (p=0.004, 50.6 Gy *versus* 56.2 Gy) and bladder dose (p=0.017, 48.5 Gy *versus* 61.5 Gy). There were no difference in scores on masculinity and sexuality function. In conclusion, this retrospective study indicates that dose escalation up to 76 Gy employing the BeamCath® catheter technique does not influence either rectal or bladder toxicity, compared with conformal technique of 70 Gy.

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SPECIFIC NUTRIENT SYNERGY IN THE TREATMENT OF LEUKEMIA

S. Harakeh[1,2], J. Khalife[1], M. Diab-Assaf[1], E. Baydoun[1], A. Niedzwiecki[2] and M. Rath[2]

[1]Biology Department, American University of Beirut, Beirut, Lebanon;
[2]Dr. Rath Research Institute, Santa Clara, CA, USA

The effects of a specific nutrient synergy (SNS), consisting of a combination of antioxidants, vitamins and amino-acids (three of its major components being ascorbic acid (AA), epigallocatechin gallate (EGCG) and L-lysine) were investigated on proliferation and induction of apoptosis

using non-cytotoxic concentrations against HTLV-I-positive and -negative malignant T-cells. In addition, the effects of SNS, AA, EGCG and L-lysine were evaluated on the activity, transcriptional and translational levels of the two important gelatinases, metalloproteinases-2 and -9, as well as on the NF-κB pathway and Tax production in HTLV-I-positive cell lines. The results indicated that the SNS had a more potent anti-proliferative effect than its individual ingredients and more pronounced induction of apoptosis in both HTLV-I-positive and -negative malignant T-cells. The SNS also inhibited extravasation by significantly down-regulating MMP-2 and -9 activity, production and expression as was shown by zymography, Western blotting and RT-PCR. Moreover, the SNS inhibited the translocation of the p65/p50 subunits to the nucleus in a dose-dependent manner as well as Tax production in HTLV-I-positive malignant T-cell lines.

In conclusion, the results indicate that the SNS could constitute a potential anti-invasive treatment in adult T-cell leukemia and related diseases. Based on the effectiveness of the SNS in targeting common critical mechanisms involved in cancer and its minimal cell toxicity, as compared to pharmaceutical drugs, clinical investigations are highly recommended.

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THE ANTIOXIDANT AND ANTI-PROLIFERATIVE ACTIVITY OF *ORIGANUM MAJORANA* AND *OLEA EUROPEA* EXTRACTS USING LEUKEMIC CELL LINES

R.M. Abdel-Massih[1], R. Fares[1], E. Baydoun[2] and S. Harakeh[3]

[1]Department of Biology, University of Balamand, Al-Koura;
[2]Department of Biology, American University of Beirut, Beirut, Lebanon;
[3]Dr. Rath Research Institute, Santa Clara, CA 95050, USA

Plant extracts from *Origanum majorana* and *Olea europea* were evaluated for their antiproliferative and antioxidant effects using human T-lymphoblastic leukemia cell lines (CEM and Jurkat). Cytotoxicity of various concentrations of plant extracts were examined using non-radioactive cytotoxicity assay and the IC_{50} was calculated. Olive leaves were found to be more cytotoxic than majoram since they have lower IC_{50}. At noncytotoxic concentrations, the viability of cells decreased with the increase in concentration of plant extract as determined by the WST-1 proliferation kit. The antiproliferative effect was further analyzed using the [^{3}H]-thymidine incorporation method and was dose dependent. To investigate whether cell death was due to apoptosis, cells were stained with annexin V-FITC and PI. Flow cytometry showed that majoram and olive leaves extracts induced apoptosis. The antioxidant activity of plant extracts was studied using the DPPH scavenging method. Majoram (IC_{50}=0.03 mg dry weight) exhibited a stronger scavenging activity than olive leaves (IC_{50}=0.1 mg dry weight).

The conclusions from this study suggest that majoram and olive leave extracts exhibit antiproliferative effect on malignant T-cells and have a high antioxidant activity. For that they merit further investigation as a potential therapeutic agent.

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ANGIOGENESIS *VERSUS* LYMPHANGIOGENESIS IN LUNG CANCER; A CRASH TEST DEFINING THE BEGINNING OF A NEW ERA

Georgia Hardavella[2,4], Evdokia Arkoumani[1], Yiotanna Dalavanga[3], Stavros H. Constantopoulos[2] and Dimitrios Stefanou[1]

[1]Department of Pathology, University of Ioannina, Medical School, Ioannina;
[2]Department of Pneumonology, University of Ioannina, Medical School, Ioannina;
[3]Department of Anatomy, Histology and Embryology, University of Ioannina, Medical School, Ioannina;
[4]Respiratory Medicine Department, Medical School, Athens University, Athens, Greece

Introduction: Tumor angiogenesis is a highly regulated process influenced by the host microenvironment and mediators. VEGF and its receptors (VEGF-R1-Flt-1, VEGF-R2-KDR/Flk-1) are good markers of vascular proliferation but their expression and relevance to tumor spread and their correlation with lymphangiogenesis and patient outcome in lung carcinomas is yet to be defined. *Aim:* To investigate the expression of angiogenesis and lymphangiogenesis in lung carcinomas (non small cell-NSCLC and small cell-SCLC), to study their interrelationship and prognostic influence and to compare it with other carcinomas studied in the literature. *Materials and Methods:* One hundred and forty six patients with lung carcinoma (96 NSCLC and 50 SCLC) were rectospectively reviewed. Tumor specimens were stained for VEGF and CD105 (DBS California-Menarini Hellas). VEGF expression, intratumoral lymphatic microvessel density (ILMVD) and lymphatic invasion were determined and thorough study of inpatient medical records followed. *Results:* VEGF and CD105 expression were significantly associated with the basic histological type of lung neoplasms (df=2, p=0.002 and p=0.04 for NSCLC and SCLC respectively). High ILMVD was detected in 37/50 SCLC and in 84/96 NSCLC. In NSCLC, VEGF-R1-Flt1 and VEGF-R2-Flk1 were associated with the stage (p=0.026, p=0.005) and the latter was also associated with metastasis (p=0.021).

Significant association was found between CD105 and the stage of the disease (NSCLC x^2=19,5, p=0.003 and SCLC x^2=8,4, p=0.004). CD105 expression was also associated with the presence of metastasis in both NSCLC (p=0.003) and SCLCs (p=0.05). In contrast, no significant correlation was assessed between VEGF and the clinical parameters mentioned above in both SCLC and NSCLC. *Conclusion:* There is a direct association between VEGF and lymphangiogenesis in NSCLC. SCLCs present a comparatively lower VEGF expression and increased lymphangiogenesis, thus displaying a different behavioral pattern. CD105 expression is strongly associated with the clinical parameters studied and could be capable of constituting a significant prognostic role in the overall aspect of lung cancer prognosis.

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TUMOR M2 PYRUVATE KINASE: CLINICAL APPLICATIONS IN GASTRIC CANCER

P.D. Hardt

Third Medical Department, Giessen and Marburg University Hospital, Giessen, Germany

In tumor cells, a dimeric isoenzyme of pyruvate kinase, termed Tumor M2 pyruvate kinase (Tumor M2PK) is overexpressed. Using specific antibodies, histological studies showed that this particular isoenzyme can be detected in huge amounts in almost every malignant tissue. Several years ago, an ELISA was developed to measure Tumor M2PK levels in EDTA plasma. Later the ELISA was slightly modified to be used in stool samples.

While a lot of attention has been paid to the possible role of fecal Tumor M2PK measurements in the screening for colorectal cancer recently, only a very few studies dealt with other cancer entities of the gastrointestinal tract. In gastric cancer, which is one of the most frequent cancer diseases in the world, no reliable tumor markers have been established as yet. If any, CEA, Ca19-9 and Ca 72-4 have been discussed; however, the reported sensitivities were only about 40%. In contrast to this, Tumor M2PK measurements reported a sensitivity of about 60% at 95% specificity. Thus, determination of this marker in EDTA does provide an instrument to be used in the follow-up and therapy control of gastric cancer cases which is superior to the markers previously used. Furthermore, gastric cancer patients do also show elevated levels of Tumor M2PK in the feces. This is true for the "classical" assay that has been developed for screening purposes in colorectal cancer but also for another essay using a different marker combination that appears to be more specific for gastric cancer. In populations with a high prevalence of gastric cancer, fecal Tumor M2PK testing might be a means for screening.

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THE PROLIFERATIVE CELL NUCLEAR ANTIGEN OF SEQUENTIAL PROGRESSION IN COLORECTAL ADENOMA-DYSPLASIA-CARCINOMA TRANSITION

Revekka Harisi[1,2], Janos Weltner[2], Balazs Jaray[3], Krisztina Morvay[2], Peter Kupcsulik[2], Laszlo Harsanyi[2], Tamas Winternitz[2], Zsuzsanna Schaff[3] and Andras Jeney[1]

[1]First Institute of Pathology and Experimental Cancer Research, [2]1st Department of Surgery, [3]Second Institute of Pathology, Semmelweis University, Faculty of Medicine, Budapest, Hungary

Introduction: The role of proliferating cell nuclear antigen (PCNA) to colorectal tumorigenesis has not yet been settled; in fact, while some authors reported a relevant role for PCNA tumor expression as a direct indicator of poor survival, others have not confirmed this association. *Aim:* Examination of the shift through the adenoma-carcinoma progression sequence of human colon and rectum in context with the expression difference of proliferating cell nuclear antigen between adenoma-carcinoma and the adjacent normal mucosa (PCNA-ED). *Materials and Methods:* 178 patients with colorectal polyp-adenoma and/or colorectal carcinoma underwent endoscopic polypectomy or surgical resection. The PCNA expression was examined in 28 non-neoplastic epithelial polyps, 82 neoplastic adenomatous polyps showing different degrees of dysplasia, 12 *in situ* carcinomas, 6 malignant polyps, 66 colorectal adenocarcinomas and their adjacent mucosa specimens. The PCNA protein levels were determined by immunoblot analysis quantified by densitometry. Logistic regression was used to examine the predictive role of PCNA on adenoma-carcinoma sequence. *Results:* The PCNA expression was detectable in the visibly normal mucosa of 69% of patients with polyps. This alone is a proliferative zone, but does not mean bad prognosis. Moving further in the adenoma-carcinoma sequence the PCNA-ED was more frequent. This kind of difference was never found hyperplastic and hamartomatous polyps or inflammatory pseudopolyps. Low difference was found in 2% of tubular and in 6% of tubulo-villous adenomas, and further 6% of the latter had high difference. In case of villous adenomas the degree of dysplasia well correlated with the frequency of difference. In mild dysplasia the expression difference was found in 3% of the cases, in moderate it was in 21% and in severe dysplasia the difference was found in 55% of the cases. The adenoma size and the increase of PCNA-ED among the patients were linearly correlated (R^2=0.96 for tubular and tubulovillous and 0.99 for villous adenomas by linear regression). The increasing severity of the dysplasia, the increasing size of the polyp and the more frequent detection of PCNA-ED among the patients were strongly correlated values. The distribution of PCNA-ED along with the recurrence was highly statistically significant

($p<<0.005$). In all patients with high PCNA-ED recurrence was developed within the first year. In development of the one- to three-year recurrence the low PCNA-ED played the dominant role, while in development of the one-year recurrence the high PCNA-ED was more dominant. PCNA-ED has been found in 57% of *in situ* carcinomas grown on base of a polyp, and in 69% of the malignant polyps. In adenocarcinomas, the Dukes classification paralleled well with PCNA-ED, in contrast with the only in tumor measured PCNA expression. *Conclusion:* These results indicate that PCNA expression difference between normal and altered tissue progressively increased along the sequence from normal mucosa via low grade, middle grade, and high grade dysplasia, adenoma to advanced cancer. The PCNA-ED is one parameter that contributes to the definition of the degenerative risk and allows selection of patients with high expression difference for constant monitoring.

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PUTATIVE MOLECULAR TARGETS FOR ANTIMIGRATORY TUMOR THERAPY

Revekka Harisi, Ferenc Timar, Julia N. Olah, Istvan Kenessey, Istvan Babo, Tibor Szarvas, Gabor Pogany, Sandor Paku and Andras Jeney

First Institute of Pathology and Experimental Cancer Research, Semmelweis University, Faculty of Medicine, Budapest, Hungary

Background: Metastasis – the spread of tumor cells from the primary site to distant organs – represents a major pathobiological event in tumor progression, and decides the outcome of the malignant disease. Although paramount progress has currently been witnessed in the control of the primary tumors, it is a great challenge to develop drugs, showing specific action against tumor progression. Metastasis is a multistep process involving shedding of tumor cells from the primary site, migration and attachment in a novel microenvironment, where they could settle down and form a viable growing cell population. Antimetastatic agents not necessarily act on cell proliferation; rather one of the above mentioned pathobiological events must be affected. *Purpose:* For the identification of chemical compounds with predominant action on migration relative to proliferation, we decided to recognize certain molecules as promising targets, specific to metastatic process. To this end appropriate *in vitro* biological assay has been elaborated. *Materials and Methods:* As our previous studies provided further evidence for the contribution of matrix metalloproteinases (MMPs), proteoglycans and integrins in the invasive growth, compounds inhibiting these molecules were selected as promising antimetastatic agents. To investigate tumor cell migration, three dimensional culture of human osteosarcoma cells (OSCORT) and human fibrosarcoma cells (HT-1080) were applied. Tumor cells grown in monolayer were overlaid with matrigel. After separating the cells remaining in the monolayer from the cells migrating into the matrigel, the invasive behavior of the tumor cell population could be assessed. In addition, tumor cell migration in certain cases was measured in Boyden chamber. *Results:* Invasive growth of osteosarcoma cells was stimulated in the presence of heparan sulfate proteoglycan both in three-dimensional culture and Boyden chamber. Hexyldeoxyuridine a potent inhibitor of heparan sulfate proteoglycan reduced the invasive growth of tumor cells. MMP-9 showed high activity in slow-growing HT-1080 culture with elevated migratory behavior. Antisense oligonucleotide against MMP-9 inhibited both MMP-9 and tumor cell migration. Borrelidin analogs showed a selective antimigratory activity as well, which may be related to the remarkable reduced expression of $\alpha v\beta 3$ integrin. *Conclusion:* MMP-9, heparan sulfate proteoglycan and integrins showed close relation with the migratory potency of OSCORT and HT-1080 cell cultures. Chemical compounds inhibiting one of these molecules were able to reduce tumor cell migration. The present report summarizing preclinical studies indicates that targeted therapy against tumor metastasis could be planned against these molecules.

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A MECHANISTIC INVESTIGATION INTO THE ANTI-INVASIVE EFFECTS OF OLIVE OIL PHENOLICS

Y.Z.H-Y Hashim[1], I.R. Rowland[2], M. Servili[3], M.J. McCann[4], D. Berrar[5] and C.I. Gill[6]

[1]UCD Institute of Food and Health, University College Dublin, Belfield, Dublin 4, Ireland;
[2]Department of Food Biosciences, University of Reading, Whiteknights, PO Box 226 Reading RG6 AP UK;
[3]Dipartmento di Scienze degli Alimenti, Sezione di Tecnologie e Biotecnologie degli Alimenti, Via S. Costanzo, 06126 Perugia Italy;
[4]Food Metabolism and Microbiology, Food & Textiles Group, AgResearch Limited, Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand;
[5]System Biology Research Group, Centre for Molecular Biosciences, School of Biomedical Sciences and
[6]Northern Ireland Centre for Food and Health (NICHE), University of Ulster (Coleraine), Cromore Road, Coleraine, Northern Ireland, UK BT52 1SA

Recently, certain major phenolics from virgin olive oil have been shown to modulate cellular pathways involved in

carcinogenesis, such as cell cycle, apoptosis, invasion and metastasis. Invasion is an important feature of metastasis and it comprises adhesion, degradation of basement membrane *via* proteolytic enzyme activity; and cell migration/motility. In this *in vitro* study, we focused on the anticancer effects of phenolics from virgin olive oil at the invasion level using HT115, a metastatic adenocarcinoma cell line. Olive oil phenolics extract (OVP) inhibited cell invasion in the Matrigel invasion assay but not migration through polyethylene membrane devoid of the reconstituted basement membrane. OVP also inhibited or reduced adhesion on collagen type IV and spreading on fibronectin. Interestingly, OVP was observed to inhibit adhesion and/or cause detachment of adhering cells in the assays performed. This anti-adhesion effect is proposed to be a genuine effect since OVP was not cytotoxic at the range tested (0-25 μg/ml) and we observed that OVP caused differential effects on HT115 cell surface integrin expression. Based on the clustering and pathway/functional genomics analysis on gene expression data, we propose that OVP interferes with the pathways that lead to detachment of cells from substrate. In conclusion, results from this study suggest that OVP inhibited integrin-regulated adhesion, spreading and invasion but not migration of HT115 cells. These studies revealed novel anticancer targets of phenolics from virgin olive oil, which may aid understanding in prognosis, prevention, as well as therapy of cancer at the invasion and metastasis level.

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REFINEMENT AND ANIMAL WELFARE IN CANCER MODELS

Jann Hau

Department of Experimental Medicine, University and University Hospital of Copenhagen, Denmark

Laboratory animal models have been essential for understanding tumour biology, for development and testing of drug therapies, and for risk assessments of potential carcinogens. Animal models remain pivotal for studies of biological mechanisms involved in the development of cancer and for studies of tumours growing *in vivo*. Scientists have moral and legal obligations for the welfare of the animals in their care during experimentation, and proper consideration should always be given to the 'three R's' (replacement, reduction, and refinement). When animals are necessary to address a particular question in oncology, pain and distress must be minimised, and avoidable pain is unacceptable. Studies of experimentally induced neoplasia present particular problems, and scientists should make every effort for implementation of the earliest humane endpoints possible to minimise the adverse effects on the animals. Death as an endpoint should obviously no longer be accepted, and researchers should be encouraged to introduce the earliest possible endpoints and to disseminate the information by publishing improvements with respect to refinement and animal welfare score sheets in experimental protocols.

Continuous refinement of experimental protocols resulting in the introduction of the earliest achievable endpoints requires competence, commitment and collaboration of scientists and all staff associated with animal care and animal experimentation. All staff should understand their individual responsibilities, and an unambiguous chain of communication and accountability should be established. This allows immediate action to address animal welfare issues that may arise as a consequence of experimental protocols.

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THE EXPRESSION OF EIF3, LARGE SUBUNIT (P150) IN HUMAN GLIOBLASTOMA

Johannes Haybaeck[1,2], Christoph Schindler[1], Gilbert Spizzo[3], Thomas Brunhuber[4], Georg Schäfer[4], Sabine Spiegl-Kreinecker[5], Johannes Fischer[6], Serge Weis[1] and Peter Obrist[1]

[1]Laboratory of Neuropathology, Department of Pathology and Neuropathology, State Neuropsychiatric Hopsital Wagner-Jauregg, Linz, Austria;
[2]Institute of Neuropathology, Department of Pathology, University Hospital Zürich, Switzerland;
[3]Division of Hematology and Oncology, and
[4]Department of Pathology University of Innsbruck, Innsbruck, Austria;
Departments of [5]Theoretical Neurosurgery and
[6]Neurosurgery, Wagner Jauregg Hospital, Linz, Austria

Glioblastoma multiforme (GBM) is an aggressive brain tumour associated with poor prognosis. Despite radio- and chemotherapy, survival is short. EIF3 is a multi-subunit complex that plays a central role in the translation initiation pathway. The large subunit of eIF3 that includes p150 is regarded as a key player in translation initiation and mediates most of its activities. We investigated the expression of p150 by immunohistochemistry in 46 patients with glioblastoma. Moreover primary cell lines were analysed for p150 protein expression by Western blot techniques. P150 expression was mostly localised in the cytoplasm with strongest expression in tumour giant cells. Overexpression of p150 was found in 34 tumour samples. Modern therapeutic approaches, for example Rapamycin or its analogues, may target elongation and initiation factors of protein synthesis, and thus could lead to a global cellular down-regulation, including p150 as shown for the mTor pathway.

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P150 - EIF3 LARGE SUBUNIT - PROTEIN OVEREXPRESSION IN BREAST CANCER

J. Haybaeck[1], G. Spizzo[2], T. Brunhuber[2*], G. Schaefer[2], W. Salvenmoser[3], R. Baenziger[4], F. Bachmann[4], M. Burger[4] and P. Obrist[2*]

[1]Institute of Neuropathology, Department of Pathology, University Hospital Zürich;
Departments of [2]Pathology and [3]Zoology, University of Innsbruck, Austria and
[4]Friedrich Miescher Institute, Basel, Switzerland
*Peter Obrist and Thomas Brunhuber are now in the Laboratory of Pathology, Hospital Zams, 6511 Zams, Austria

P150, a protein with an apparent molecular weight of 150 kDa, was isolated from virally and oncogene transformed mouse cell lines, partially purified and cloned. P150 belongs to the elongation initiation factor 3 complex, which consists of 10 subunits. It was shown to be part of the large subunit complex, described as being a 160 to 180 kDa protein complex. It is supposed to be a molecular parameter in predicting disease progression in cervical and oesophageal cancer. We describe the distribution of P150 in normal and neoplastic breast tissue and try to elucidate the role of the eIF3 complex during tumorigenesis. Therefore 43 breast tissue samples were examined, including benign and neoplastic tissues and compared with each other by the use of immunohistochemistry and Western blotting using a polyclonal chicken anti-p150-antibody. Strong overexpression in breast cancer was found in nearly all cases. Exceptionally, in one case of squamous cell carcinoma, an inverse reaction was found. In contrast to neoplastic tissues, the adjacent normal one revealed a slight inhomogenous positivity. The role of p150 as part of the eIF3 complex during tumorigenesis is strictly related to a selective protein synthesis in tumor cells. It mainly participates in the deregulation of the translation process by interaction with eIF4E, eIF2 and with hPrt1, and also with eIF5.

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HEPATOCYTE-SPECIFIC LYMPHOTOXIN EXPRESSION CAUSES CHRONIC HEPATITIS INDUCED HEPATOCELLULAR CARCINOMA IN *TNFR1*$^{-/-}$ BUT NOT IN *RAG1*$^{-/-}$ OR IKK$\beta^{\Delta HEP}$ MICE

Johannes Haybaeck[1*], Mathias Heikenwalder[1*], Nicolas Zeller[1*], Monika Julia Wolf[1], Juliane Bremer[1], Achim Weber[2], Michael Kurrer[3], Ulrich Wagner[4], Manfred Kopf[5], Michael Karin[6] and Adriano Aguzzi[1]

[1]Department of Pathology, Institutes of Neuropathology and [2]Clinicial Pathology, University Hospital Zurich;
[3]Department of Pathology, Cantonal Hospital Aarau;
[4]Functional Genomics Centre Zurich, University Zurich;
[5]Institute of Integrative Biology, Molecular Biomedicine,Swiss Federal Institute of Technology (ETH), CH-8952 Zurich, Switzerland;
[6]University of California, San Diego and University of California, Los Angeles, USA
*These authors contributed equally to this work.

Hepatocellular carcinoma (HCC), the most common liver cancer, is mainly induced by chronic hepatitis. Lymphotoxin (LTαβ) was recently demonstrated to be up-regulated in livers of patients suffering from virus-induced hepatitis and HCC. We generated transgenic mice with liver-specific expression of LTαβ (AlbLTαβ). Characteristic morphological features of chronic portal and lobular hepatitis were detected in livers from transgenic mice at the age of 6-9 months, preceded by hepatocyte-specific expression of chemokines (*e.g.* IP10, CXCL1, CCL2). Elevated serum levels of aminotransferases starting from 8 weeks of age indicated liver damage in AlbLTαβ mice. Remarkably, at an age of ≥300 days, 30% of transgenic mice developed HCCs. Hepatitis and HCC genesis was fully prevented in AlbLTαβ mice backcrossed to *rag1*$^{-/-}$ and Ikk$\beta^{\Delta hep}$ mice. In contrast, *tnfr1*$^{-/-}$ mice developed hepatitis and HCC. Here, we describe a new model of chronic hepatitis-induced HCC. Transcriptome analysis at various stages of hepatitis and HCC development reveals many similarities to virus-induced hepatitis and HCC in humans and enables us to dissect signalling events in AlbLTαβ, AlbLTαβ × Ikk$\beta^{\Delta hep}$ and AlbLTαβ × *tnfr1*$^{-/-}$ mice. Therefore, LTαβ expression by hepatocytes suffices to induce chronic hepatitis causing HCC in a lymphocyte- and IKKβ-dependent, but TNFR1-independent manner, rather than directly acting as an oncogene.

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LRIG PROTEINS AS REGULATORS OF GROWTH FACTOR SIGNALING

Camilla Holmlund, Wei Yi, Roger Henriksson and Håkan Hedman

Department of Radiation Sciences, Umeå University, SE-90187 Umeå, Sweden

Receptor tyrosine kinases are implicated in the etiology of many cancers. For example, in glioblastoma multiforme the epidermal growth factor receptor- (EGFR-) gene is frequently amplified and mutated to yield high levels of constitutively active receptors with prolonged half-lives. In a search for endogenous inhibitors of EGFR signaling, we identified the integral membrane protein, leucine-rich repeats and immunoglobulin-like domains 1 (LRIG1). Subsequently, we and others have shown that LRIG1 suppresses the oncogenic receptor tyrosine kinases EGFR, ERBB2, MET,

and RET. LRIG1 is down-regulated in various neoplasms, including cervical and renal cell carcinoma. We also identified two human LRIG1 paralogs, LRIG2 and LRIG3. The LRIG proteins show differential subcellular localization in normal and pathological tissues, which seems to have clinical implications. Perinuclear LRIG protein localization is associated with good survival of astrocytoma patients, whereas, cytoplasmic LRIG2 expression was associated with poor survival of oligodendroglioma patients. LRIG1 is subject to proteolytic processing and the resulting fragments seemed to have biological activities. Taken together, the LRIG proteins seem to be important regulators of growth factor signaling with implications for patient survival in various malignancies.

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A MOUSE MODEL OF NEUROFIBROMATOSIS-1 OPTIC GLIOMA: A PRECLINICAL TOOL FOR ANTI-ANGIOGENESIS RESEARCH?

Balazs Hegedus[1], Debasish Banerjee[2], Arie Perry[3], Joshua B. Rubin[4], Joel R. Garbow[2] and David H. Gutmann[1]

Departments of [1]Neurology, [2]Radiology, Division of [3]Neuropathology and [4]Pediatrics, School of Medicine, Washington University in St. Louis, St. Louis, MO, USA

Individuals affected with the neurofibromatosis 1 (NF1) are prone to develop tumors of the nervous system, including optic pathway gliomas (OPG). These tumors are classified as grade I pilocytic astrocytomas, characterized by low cellularity and rare mitotic figures. Despite their benign behavior in general, these gliomas often exhibit increased angiogenesis. Importantly, the development of prechiasmatic and chiasmatic optic gliomas has been observed in a genetically engineered mouse (GEM) model with inactivation of the neurofibromatosis-1 (*Nf1*) tumor suppressor gene in glial cells. Interestingly, the natural history and morphology of these GEM tumors was similar to their human counterparts. Furthermore, increased angiogenesis has been found during OPG development as evidenced by an increased blood vessel density. We validated this Nf1 optic glioma model using conventional chemotherapy (temozolomide) currently used for children with low-grade glioma and showed that treatment resulted in reduced proliferation and increased apoptosis of tumor cells *in vivo* as well as reduced tumor volume. Collectively, these findings indicate that this unique Nf1 GEM optic glioma model might be a potent tool for preclinical assessment of novel anti-angiogenic therapies.

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ROLE OF HYALURONAN-CD44 COMPLEXES IN REGULATION OF GROWTH FACTOR RECEPTOR ACTIVITY AND IN TUMOR PROGRESSION

Paraskevi Heldin, Berit Bernert, Spyridon Skandalis, Evgenia Karousou, Helena Porsch and Carl-Henrik Heldin

Ludwig Institute for Cancer Research, Uppsala University, Biomedical Center, Box 595, S-751 24 Uppsala, Sweden

Introduction: The interactions between cells and the host microenviroment influences cellular behavior. Hyaluronan levels are determined by synthesizing (HAS) and degrading (HYAL) enzymes in response to growth factors. The hyaluronan receptor CD44 affects cell–cell and cell–matrix interactions. *Methods and Results:* HAS activity has been shown to be important for the maintenance of the malignant and invasive phenotype of the Hs578T breast cancer cells using specific siRNAs, we showed that HAS interacts with HYAL and the hyaluronan receptor CD44 to promote the aggressive character of breast cancer cells. Furthermore, using a 3D collagen matrix assay we studied in real-time the mechanisms by which CD44-hyaluronan interactions affect transendothelial migration; peritumoral hyaluronan is important for the early adhesion of tumor cells to endothelial cells. Recently, we investigated the downstream signaling pathways through which PDGF-BB stimulates hyaluronan synthesis using inhibitors of different signaling pathways, we showed that the Erk MAP kinase and PI3 kinase signaling pathways are necessary for the regulation of hyaluronan synthesis by PDGF-BB and that hyaluronan affects the mitogenic response to PDGF-BB. Recent data revealed that tissue hyaluronan content can be modulated through ubiquitinylation and possibly oligomerization of HAS enzymes. In another line of research, we demonstrated that CD44 forms a complex with both the PDGF β-receptor and TGFβ type I receptor; hyaluronan-activated CD44 suppressess the PDGF-BB-mediated activation of the PDGF β-receptor and TGFβ-mediated activation of Smad2. *Conclusion:* Hyaluronan favors the malignant phenotype in malignancies and modulates receptor tyrosine kinase activity.

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SINGLE OR COMBINATIONS OF TUMOR MARKERS IN CERVICAL CANCER – CORRELATION TO PROGNOSIS, SERUM PROGESTERONE AND ESTRADIOL, SMOKING AND ORAL CONTRACEPTIVE USE

Dan Hellberg

Center for Clinical Research, Falun, Sweden

Background: Expression of a single tumor marker is rarely clinically useful in any cancer type. There is an increasing interest in using panels of tumor markers to increase prognosis prediction, differential diagnosis and choice of therapy. *Materials and Methods:* One hundred and thirty women with

squamous epithelial cervical cancer had pre-treatment progesterone and estradiol, and a complete history including smoking habits and previous oral contraceptive use. A panel of 13 novel and traditional tumor markers were selected and analysed by immunohistochemistry. Follow-up was at least 10 years. *Results:* Expression of six tumor markers was significantly associated with 10-year survival, after adjustment for clinical cancer stage, these were LRIG1, p53, and $CD4^+$, (favourable prognosis). LRIG2, COX-2 and c-myc expressions were associated with poor prognosis. LRIG1 and LRIG2 will be discussed elsewhere at the conference. With Cox regression, none of the other tumor markers were significantly associated with prognosis. When combinations of tumor markers with different functions in carcinogenesis were combined with Cox regression and adjusted for stage, four combinations significantly predicted prognosis, while another four combinations were of borderline significance (p=0.05). Expression of p53 was strongly reduced in smokers. C-myc expression was correlated to increased serum progesterone levels, while p53 expression was reduced. *Conclusion:* Combinations of tumor marker expression were more reliable in predicting prognosis than a single marker. Our results also provide biological explanations behind the role of risk factors in cervical cancer. The results of some other studies will be discussed.

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THE ROLE OF GENISTEIN DERIVATIVES STRUCTURE AND SOME MOLECULAR PROPERTIES IN THEIR INTERACTION WITH MEMBRANES AND ANTIOXIDANT ACTIVITY

Kamila Środa[1], Jadwiga Maniewska[1], Krystyna Michalak[1], Grzegorz Grynkiewicz[2], Wiesław Szeja[3] and Andrzej B. Hendrich[1]

[1]Departement of Biophysics, Wrocław Medical University, ul. Chałubińskiego 10, 40-368 Wrocław;
[2]Pharmaceutical Research Institute, ul. Rydygiera 8, 01-793 Warszawa;
[3]Departement of Organic, Bioorganic Chemistry and Biotechnology, Silesian Technical University, ul. Krzywoustego 4, 44-100 Gliwice, Poland

Many of the biological effects exerted by flavonoids, *e.g.* antitumoral, anti-inflammatory, anti-ischemic, cardioprotective, are believed to come from the antioxidant activity of these compounds. As was shown in numerous experiments, the antioxidant capacity of flavonoids depends mostly on their structure (*i.e.* number and positions of hydroxyl groups) but also on some other factors such as type of oxidative stress inducer used or ability of certain flavonoid to intercalate into lipid bilayers (in the case of lipid peroxidation). According to present knowledge, flavonoid molecules present in the membrane could directly scavenge ROS (reactive oxygen species) but also, due to the modulation of membrane properties, they can reduce the rate of ROS diffusion in membrane and thus reduce lipid oxidation.

In present work, we studied the interactions of newly synthesized benzyl and glycosylated genistein derivatives with lipid membranes. Using microcalorimetry, we found that all studied compounds interact with lipids and alter the thermotropic properties of bilayers by decreasing the main phase transition temperature and enthalpy. For glycosylated derivatives, calorimetry also showed that increase of the length of spacer separating genistein moiety and added sugars increases the perturbation induced by these compounds. ATR-IR spectroscopy allowed us to conclude that genistein and its derivatives interact with lipid polar heads by hydrogen bonding. An increase of the number of *gauche* conformers in lipid acyl chains induced by the presence of studied polyphenols was also recorded. Both calorimetric and infrared spectroscopic experiments confirmed that polar head region as well as the hydrophobic interior of the bilayer are affected by the presence of flavonoids. Studying the influence of genistein derivatives on the calcein efflux from liposomes, we found that all compounds are more active than parent genistein but glycosylated derivatives permeabilize membranes to a greater extent than benzyl ones. Analyzing the properties of studied molecules calculated by computer-aided modeling, we noticed that among ovality, octanol-water partition coefficient, polarizability, E_{HOMO}, E_{LUMO} and dipole moment, only the latter property correlates with the order of permeabilizing potency of benzyl genistein derivatives. Further analysis of computer modeling results revealed that in fact the dipole moment of an added benzyl ring together with its substitutions (fluorine, chloride, cyanine and methoxy) is the factor determining the liposome permeability changes induced by genistein derivatives. Finally, we studied the antioxidant activity of these compounds (against lipid peroxidation) and the relation between the effects exerted by the studied derivatives on lipid membranes and their antioxidant potency is discussed.

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TUMOR STEM CELLS IN GLIOMAS: CLINICAL IMPACT OF THE STEM CELL MARKER CD133 AND THERAPEUTICAL STRATEGIES

C. Herold-Mende[1], B. Campos[1], F. Wang[1], F.Zeppernick[1], R. Ahmadi[1], W. Roth[2], P. Lichter[2], A. Unterberg[1] and B. Radlwimmer[2]

[1]Department of Neurosurgery, University of Heidelberg, [2]DKFZ Heidelberg, Germany

A considerable amount of evidence has been gathered supporting the existence of tumor stem cells in a variety of

cancers. Glioma-derived tumor stem cells (GTSC) can be enriched by the stem cell surface antigen CD133. Conversely, a controlled, drug-induced depletion of the CD133-positive GTSC pool could have profound therapeutic implications. Retinoids such as all-*trans* retinoic acid (ATRA) have been shown to induce differentiation of GTSCs *in vitro*. However, it remains unknown whether tumor growth-relevant features of these cells are affected.

We analyzed expression of CD133 in 95 gliomas of various grade and histology by immunohistochemistry. Staining data were correlated with patient outcome. Furthermore, several GTSC lines with high CD133 content were established to investigate ATRA-induced differentiation and potential effects on tumor growth-relevant properties. Proliferation was monitored by BrDU-incorporation assay and CD133 content by FACS-analysis. Impact of differentiation on angiogenic capacity of GTSCs was measured by quantification of angiogenic cytokines and assessed in a HUVEC-based tube formation assay. Potential effects on GTSC invasiveness were studied in a 3D-collagen invasion model. Finally, we studied whether *in vitro* effects could be confirmed *in vivo* using a NOD/SCID-mouse xenograft model.

By multivariate survival analysis, both the proportion of CD133-positive cells and their topological organization in clusters were significant ($p<0.001$) prognostic factors for adverse progression-free (PFS) and overall survival (OS). Furthermore the proportion of CD133-positive cells was an independent risk factor for tumor regrowth and time to malignant progression in WHO II and III tumors. Supporting these clinical data, we present functional evidence that GTSCs exposed to ATRA lower the expression of CD133 in favor of incremented expression of lineage markers. This is accompanied by a significantly reduced secretion of VEGF and bFGF, as well as a significantly lowered angiogenic activity following differentiation. Additionally, we show that differentiation elicits strong anti-invasive effects, reducing invasion of GTSCs accompanied by a down-regulation of invasion-related MMP2 protein. Finally, we report that xenografted tumors of differentiated GTSCs are significantly smaller and less invasive than undifferentiated GTSC tumor xenografts. Correspondingly, animals bearing differentiated cells show both significantly better PFS and OS than mice with GTSC xenografts.

These findings constitute the first conclusive evidence that CD133 expression correlates with patient survival in gliomas, lending support to the current cancer stem cell hypothesis. Additionally, we present functional evidence that differentiation treatment targets the tumor-driving compartment in glioblastoma and constitutes a potential therapeutic approach in the eradication of GTSCs.

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SULFORAPHANGE ERADICATES PANCREATIC CANCER STEM CELLS BY NF-κB

G. Kallifatidis[1], V. Rausch[1], B. Baumann[2], A. Apel[1], J. Mattern[1], M. Buechler[3], A.V. Salnikov[1] and I. Herr[1]

[1]University of Heidelberg and DKFZ, Heidelberg;
[2]University of Ulm, Ulm;
[3]University of Heidelberg, Heidelberg, Germany

Emerging evidence suggests that cancer stem cells (CSCs) play a central role in the pathogenesis of pancreatic cancer. We identified CSCs in pancreatic cancer cell lines and patient tumors with a $CD44^+/CD24^-$, $CD44^+/CD24^+$, or $CD44^+/CD133^+$ phenotype and their presence correlated to high therapy resistance. Mechanistically, we observed specific binding of transactivation potent c-Rel containing NF-κB complexes in CSCs but not in non-CSCs. A compound found in broccoli, sulforaphane, prevented NF-κB binding and induced IκBa along with strong induction of apoptosis and prevention of clonogenicity. Other chemopreventive agents, gemcitabine or the death ligand TRAIL were less effective. In a xenograft model, sulforaphane strongly blocked tumor growth and combination with TRAIL, had an additive effect without obvious cytotoxicity to normal cells and stem cells. *Ex vivo*, sulforaphane abrogated resistance of primary patient tumor cells harboring CSCs. Our data suggest combination of sulforaphane with TRAIL as promising strategy for targeting of pancreatic CSCs.

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AP-1-DEPENDENT GENETIC PROGRAMS IN SKIN REMODELLING AND CANCER

Jochen Hess, Axel Szabowski and Peter Angel

Deutsches Krebsforschungszentrum, Division Signal Transduction and Growth Control, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

The transcription factor AP-1, which is mainly composed of members of the Fos and Jun protein families, participates in physiological and pathophysiological processes due to its central role as a cellular switch of genetic programs in response to extracellular signals. AP-1 subunits and their specific target genes exhibit distinct expression patterns in epidermal keratinocytes as well as mesenchymal cells. Together with impaired skin regeneration and carcinogenesis in AP-1 compromised mouse models these findings indicate that AP-1-dependent genetic programs play a pivotal role in epidermal organization, skin homeostasis and tumourigenesis. Employing genome-wide expression analysis on samples of genetically modified mouse and cell culture model systems, we are studying the function of AP-1 and its target genes within a complex dynamic network of signalling pathways implicated in the well-coordinated intercellular communication between keratinocytes, fibroblasts and immune cells. We found trans-

regulatory functions of Jun family members in the epithelial-mesenchymal crosstalk and could identify novel AP-1 target genes in dermal fibroblasts including cytokines, chemokines and growth factors. Additionally, we could identify a signalling pathway triggered by the receptor for advanced glycation end products RAGE that activates AP-1 in epithelial tumour cells and is initiated by ligands that are expressed in epithelial as well as mesenchymal cells under pro-inflammatory conditions. In the context of tumour cell invasion and malignant progression, we identified the mucine-like glycoprotein Podoplanin as a novel AP-1 target gene in epithelial tumour cells and fibroblasts.

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IN VITRO MATURATION OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS UNDER SERUM-FREE CONDITIONS IN THE PRESENCE OF ACID-TREATED GRAM-NEGATIVE BACTERIA LOADED WITH PROSTATE-SPECIFIC ANTIGEN

Bernd Hildenbrand[1], Frank Neumann[3], Dirk R. Lorenzen[2], Victoria Küttner[6], Marina Gummenscheimer[1], Stefan F. Martin[4], Michael Huber[5], Clemens Unger[1], Marina A. Freudenberg[6], Chris Galanos[6] and Marc Azemar[1]

[1]Tumor Biology Center, D-79106 Freiburg;
[2]Institute for Tumor Therapy, D-37115 Duderstadt, Germany;
[3]BIOAXXESS Technology, WR14 3SZ, Malvern, UK;
[4]University Medical Center, D-79104 Freiburg;
[5]Molecular Immunology, University Freiburg, D-79108 Freiburg;
[6]Max-Planck Institute of Immunobiology, D-79108 Freiburg, Germany

Introduction: Acid-treated gram-negative bacteria (ATB) are powerful immunostimulatory adjuvants *in vivo* and have been shown to enhance impaired immune functions in patients (1-4). As they expose lipid A on their surface they are expected to mature dendritic cells most efficiently *via* Toll-like receptor 4. Moreover, as they are non-infectious, they should serve as optimal carriers for different antigens suitable as anticancer vaccines. In this work we tested acid-treated gram-negative mutant (R-form) *Salmonella minnesota* bacteria loaded with the prostate-specific antigen (PSA) or PSA-peptides (PSAP) for their capacity to induce maturation of human monocyte-derived dendritic cells (MoDCs) under serum-free conditions. *Methods:* MoDCs were generated in serum-free medium supplemented with GM-CSF and IL-4 for 5 days and matured for 24 h with ATB (either with R-or S-(wild-type) form) loaded with the whole PSA protein or PSAP ± IFN-γ. MoDCs were phenotypically characterised by FACS and cytokine-profiled by ELISA for secreted IL-6, IL-10 and p70 (biological active) IL-12. *Results:* In particular, R-form ATB preparations loaded with PSA or PSAP induced phenotypic characteristics of mature MoDCs such as the up-regulation of CD83, CD80, CD86, HLA-DR and down-regulation of CD14. The induced expression of CD83 on MoDCs as well as their cytokine production, was higher after stimulation with ATB loaded with PSAP compared to ATB loaded with PSA. Both approaches led to higher levels of IL-12p70 and lowered IL-10 levels when IFN-γ was added to the maturation protocol. The highest ratio of IL-12p70 against IL-10 levels was observed in the presence of IFN-γ using ATB R-form preparations on their own or loaded with PSAP. *Conclusion:* ATB preparations represent suitable agents for the *ex vivo* maturation and antigen-loading of human MoDCs under serum-free conditions as exemplified for PSA and PSAP. In combination with IFN-γ all ATB preparations tested led to an enhanced T_H1-polarisation, establishing an adjuvant strategy, which should improve and facilitate future clinical protocols for cellular anticancer immunotherapy.

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DETECTION OF THERAPY-INDUCED TUMOUR DEATH BIOMARKERS IN PATIENT SERUM SAMPLES

Bernd Hildenbrand[1], Frank Neumann[2], Marc Azemar[1], Ulrich Massing[1], Clemens Unger[1] and Klaus Mross[1]

[1]Tumor Biology Center, Breisacher Str. 117, 79106 Freiburg, Germany;
[2]BIOAXXESS Technology, Malvern Hills Science Park, WR14 3SZ, Malvern, UK

Introduction: M30-Apoptosense and M65 are validated markers in CE-marked enzyme-linked immunosorbent assays

(ELISA) that detect circulating cytokeratin 18 fragments (CK18F/M30) released into the circulation during caspase-dependent (apoptotic) or total cell death, respectively, and have shown potential as biomarkers in epithelial cancers (1-3). We present data using the M30 and M65 ELISAs for investigating the caspase-cleaved neoepitope of cytokeratin 18 fragments (CK18F/M30) and/or total CK18 (M65). These might represent useful serum markers *in vivo* for drug-induced carcinoma cell death and/or tissue toxicity. The presence of liver metastases and liver injury prior to treatment may enhance the CK18 biomarker signal strength and induction pattern. Furthermore the optimal time window for serum sampling for this type of cell death biomarker analysis was evaluated. *Methods:* M30 and M65 antigen levels were measured by ELISA (4) in serum samples from 11 patients suffering from carcinomas of the breast, ovaries, oesophagus and larynx, that were taken shortly before, during and after treatment with paclitaxel as part of a pharmacodynamic clinical study. *Results:* Significant increases of serum tumor cell death biomarkers were found over pre-treatment values in 10/11 patients, which peaked or reached plateau after approx. 24 h post drug infusion. Most patients showing an increase in M65 (total CK18) serum levels also had a concomitant and comparable increase of the apoptotic M30 (caspase-cleaved CK18/CK18F) serum biomarker. No effect of the presence of liver metastases or elevated liver enzymes on baseline or the extent of treatment-induced increases in M30 or M65 serum levels were observed. The data obtained suggest that baseline M30/M65 serum values could have diagnostic potential as prognostic cancer biomarkers reflecting overall disease activity and as a predictive clinical response marker. *Conclusion:* Monitoring of serum CK18 and CK18F/M30 levels by ELISA prior, during and after chemotherapy therapy may provide a simple and minimally-invasive method for the quantitative assessment of therapy-induced antitumour activity in different carcinoma types and/or for prognostic purposes. Further validation of the prognostic and predictive value of these biomarkers is necessary.

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2-HYDROXY-OCTADECYLPHOSPHOCHOLINE ENHANCES CD83 EXPRESSION OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS MATURED WITH LPS AND IFN-γ, INHIBITING CYTOKINE SECRETION WITHOUT AFFECTING T_H1 POLARISATION

Bernd Hildenbrand[1], Dirk R. Lorenzen[2], Frank Neumann[3], Barbara Sauer[1], Ralf GRAESSER[1], Lenka A. Tylor[1], Victoria Küttner[5], Marina A. Freudenberg[5], Chris Galanos[5], Ulrich Massing[1], Clemens Unger[1] and Marc Azemar[1]

[1]Tumor Biology Center, Breisacher Str. 117, 79106 Freiburg;
[2]Institute for Tumor Therapy, Hinterstraße 53, 37115 Duderstadt, Germany;
[3]BIOAXXESS Technology, Malvern Hills Science Park, WR14 3SZ, Malvern, UK;
[4]Molecular Immunology, Biology III, University Freiburg, Stübeweg 51, 79108 Freiburg;
[5]Max-Planck Institute of Immunobiology, Stübeweg 51, 79108 Freiburg, Germany

Introduction: Lysophosphatidylcholine (LPC) is a lipid signalling messenger molecule which exhibits potent pro-inflammatory activities and can efficiently stimulate immune cells, such as macrophages or dendritic cells (DCs) (1). The *R*-configured enantiomer of 2-hydroxy-octadecylphosphocholine (R-OH), on the other hand has recently been shown to be a competitive inhibitor of cellular LPC reacylation and possess antitumor activity (2). In this study, we investigated whether R-OH could serve as a potential inhibitor of DC-mediated inflammatory processes by analyzing its influence on the maturation and/or cytokine production of human monocyte-derived DCs (MoDCs) stimulated with lipopolysaccharides (LPS) in the presence or absence of Interferon-gamma (IFN-γ). *Methods:* Immature human MoDCs were generated in serum-free medium supplemented with GM-CSF and IL-4 for 5 days and matured for additional 24 hours with LPS ± IFN-γ. R-OH or LPC (as a control) was added 24 hours before or simultaneously with LPS. Matured MoDCs were phenotypically characterised by FACS and cytokine-profiled by ELISA for secreted IL-6, IL-10 and IL12p70 (biological active). *Results:* Immature MoDCs showed enhanced up-regulation of the co-stimulatory molecules CD80 and CD86 when incubated with LPC [10-50 μM] or R-OH [10-20 μM] over a period of more than 24 hours in serum-free medium. However, compared to LPC, pre-

incubation of immature MoDCs with R-OH followed by maturation with LPS ± IFN-γ led to twice as high an up-regulation of CD83, which was associated with a strong inhibition of cytokine production. Interestingly, whereas LPC enhanced TH_2-polarisation after maturation by LPS, even in the presence of IFN-γ, pre-incubation of MoDCs with R-OH still enhanced the TH_2-polarisation when matured with LPS alone, however, it did not alter the strong T_H1-polarisation seen previously after maturation with LPS in the presence of IFN-γ (3). *Conclusion:* Pre-incubation of immature MoDCs with R-OH at non-toxic concentrations [10-20 μM] efficiently inhibits the cytokine secretion by MoDCs matured with LPS and IFN-γ without changing their IL-10/IL-12p70 ratio or T_H1-polarisation. Moreover, R-OH enhanced the up-regulation of both co-stimulatory cell surface molecules and the maturation marker CD83, which, in addition to its immune regulatory functions, may potentiate antitumor immune responses. In conclusion, these results may form the basis to explain not only the anti-inflammatory role of R-OH, but also its antitumor properties.

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FOCALIZED MULTI-THERAPIES USING AN INNOVATIVE INJECTION TECHNIQUE

E. Hiltbrand and S. Humbert

University Hospital Geneva and CERMA, Geneva, Switzerland

Targeted MULTI Therapy (TMT) primarily developed as a new thermoablative technique for cancer, is currently being extended to phlebological applications. As animal investigations have shown the safety and the efficacy of the treatment, the technique was applied successfully to humans. Besides thermoablative procedures, taking advantage of the MULTI capability of the technique, investigations are conducted in several therapeutic areas of oncology: delivery of drugs and radioactive particles under pressure directly into targeted tumours.

In this context, an experiment in which a new apoptogenic molecule with proven efficacy on cells in culture, and with the remarkable property of being irreversibly cytotoxic to human prostate epithelial cancer cells but reversibly cytostatic to human prostate epithelial normal cells (Quash G. Eur. J. Med. Chem. (2007), was successfully carried out on an animal model.

Additionally, the injection of radioactive particles with the same technique is currently being investigated on rats (European project: INBARCA). The delivery of the active agent directly into the lesion minimizes side-effects, enhances efficacy and reduces doses. The high pressure ensures an even better diffusion of the agent.

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TARGETING NITROSATIVE STRESS FOR CANCER THERAPY

David G. Hirst, Helen O. McCarthy, Jonathan Coulter, Natalie Page and Tracy Robson

Experimental Therapeutics Research Group, School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK

Nitric oxide ($NO^{\bullet}$) exhibits many of the desirable properties of an anticancer molecule. It is highly diffusible through tissues and demonstrates specificity for the tumour microenvironment. It is likely that this characteristic results from the differential production of reactive nitrogen species, particularly peroxynitrite, in tumours compared with their normal counterpart and in the downstream consequences of their generation. The effects of $NO^{\bullet}$ are highly concentration-dependent, but at high therapeutic levels it is strongly pro-apoptotic, anti-angiogenic and anti-metastatic. In addition, it has been shown to be one of the most potent radiation sensitizers known and can also enhance the cytotoxicity of some chemotherapeutic agents. However, these properties can be fully exploited only if effective strategies for delivery can be developed. At present, both $NO^{\bullet}$ donor drugs and inducible nitric oxide synthase (iNOS) gene therapy have shown impressive efficacy in experimental animal models of human cancer. We have used gene promoters that are inducible by hypoxia, radiation and the tumour microenvironment to target the expression of iNOS and hence generation of high concentrations of $NO^{\bullet}$ to the tumour volume. Twice weekly administration of this therapy in a mouse model of prostate cancer inhibited tumour growth for several months. Thus, $NO^{\bullet}$ therapy shows considerable promise as an anticancer strategy.

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AKT INHIBITOR INHIBITS CELL PROLIFERATION IN MALIGNANT FIBROUS HISTIOCYTOMA CELLS

Toshiaki Hitora, Sasaki Kanji, Osamu Nakamura, Kawaguchi Youji and Yamamoto Tetsuji

Department of Orthopaedic Surgery, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan

Introduction: The phosphatidylinositol 3-kinase (PI3K)/Akt pathway plays an important role in various cellular processes including cell growth, survival, and motility. Recently, accumulating evidence indicated that PI3K/Akt pathway plays a crucial role in tumorigenesis and tumor progression by promoting cell proliferation and inhibiting apoptosis (1). In addition, abnormal function of the PI3K/Akt pathway has been reported in many human tumors and this signaling pathway has been suggested to be a potential target for cancer chemotherapy (2). We examined the expression of Akt and the existence of PI3K/Akt signaling pathway in human malignant fibrous histiocytoma (MFH) cell lines, and the inhibitory effect of Akt inhibitor on the cell proliferation. *Materials and Methods: Cell lines and reagent*. Three human MFH cell lines (Nara-H, GBS-1, TNMY1) were used in this study. TNMY1 was previously established in our laboratory. All cell lines were grown in culture medium consisting of Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich). The cell lines were routinely maintained at 37˚C in a humidified 5% CO_2 atmosphere. Akt inhibitor X, a specific Akt kinase inhibitor was purchased from Calbiochem (San Diego, CA, USA). *The inhibitory effect of Akt inhibitor.* The cell proliferation was assayed using the MTS assay (CellTiter 96® Aqueous One Solution Cell Proliferation Assay; Promega, Madison, WI, USA). Cells were seeded in 96-well cell culture plates in culture medium with 10% FBS. After 24 hours (h), the medium was refreshed with 1% FBS containing AKT inhibitor in the indicated concentrations. After 24 and 48 h, the medium was removed and washed with phosphate-buffered saline, then refreshed with fresh medium containing MTS reagent. The optical density was measured at 490 nm using an automatic microplate reader after 2 h of further incubation. The percent age viability of each well was calculated. At least three independent cultures were performed for each study. The data were analyzed statistically using ANOVA with Fisher's PLSD *post hoc* test. *Western blotting*. Cells were pretreated for 60 min with 1% FBS containing AKT inhibitor at different concentrations. Whole cell lysates were collected for protein content, and cell lysates were separated by SDS polyacrylamide gel electrophoresis under reducing conditions. Then gels were electrophoretically transferred to PVDF membrane, and immunoblotted with anti-AKT1/2/3 antibody (Santa Cruz Biotechnology, CA, USA) and anti-phospho-AKT1/2/3 antibody (Ser 473, Thr 308; Santa Cruz Biotechnology). Bound antibodies were detected using the ECL plus Western blotting detection system (GE Healthcare Bio-Sciences, Piscataway, NJ). *Results: The effect of the Akt inhibitor:* Akt inhibitor inhibited the cell proliferation of all 3 cell lines in a dose- and time-dependent manner; 10 μM AKT inhibitor inhibited the cell proliferation of Nara-H and GBS-1 at the percent viability of 50% or less; 25 μM AKT inhibitor inhibited the cell proliferation of TNMY1 at viability of 50% or less. *Expression of Akt and phospho-Akt:* Western blotting analysis revealed that not only Akt but phospho-Akt were expressed in all cell lines under the normal condition, so it was suggested that Akt signaling pathway was always activated in all 3 cell lines under the normal condition. Phosphorylation of Akt was reduced by 25 μM Akt inhibitor in all cell lines. *Discussion:* The PI3K/Akt pathway is very important as a target of the molecular targeting therapy. In our study, Akt inhibitor showed a dose-dependent inhibitory effect on the cell proliferation of human MFH cells. Akt inhibitor reduced the phosphorylation of Akt. These results suggest that the PI3K/Akt signaling pathway exists and plays an important role in cell proliferation in MFH cells. Although further studies are needed to explore the mechanisms of cell proliferation, Akt inhibitor will be a potent chemotherapeutic agent for human MFH.

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DIALLYL DISULFIDE (DADS) INDUCES APOPTOSIS IN HUMAN CERVICAL CANCER HELA CELLS *VIA* REACTIVE OXYGEN SPECIES, ENDOPLASMIC RETICULUM STRESS AND MITOCHONDRIA-DEPENDENT PATHWAY

Chin-Chin Ho[1], Yueh-Shan Weng[2], Jai-Sing Yang[3], Hsu-Feng Lu[4] and Jing-Gung Chung[2]

[1]Department of Nursing, Central Taiwan University of Science and Technology, Buzih District, Taiwan;
Departments of [2]Biological Science and Technology and [3]School of Pharmacology, China Medical University, Taichung, Taiwan;
[4]Department of Clinical Pathology, Cheng Hsin Rehabilitation Medical Center, Taipei, Taiwan, ROC

Although many studies have shown that diallyl disulfide (DADS), a substance found in garlic, induced apoptosis in various human cancer cells, the molecular mechanisms of apoptosis induced by DADS in human cervical cancer cells are not clear. In this study, we tested DADS for induction of

apoptosis in human cervical cancer HeLa cells. DADS induced morphological changes and reduced the percentage of viable HeLa cells in a dose- and time-dependent manners. Flow cytometric analysis demonstrated that DADS induced apoptosis in HeLa cells. DAPI staining showed morphological features of apoptosis in HeLa cells treated with 50 μM DADS for 24 h. ApopTag assay demonstrated DNA fragmentation in apoptotic cells. DADS induced apoptosis through the production of reactive oxygen species and Ca^{2+}, and induced the abrogation of changes in mitochondrial membrane potential ($\Delta\psi m$) and cleavage of Bid protein (t-Bid). DADS also promoted the activities of caspase-3 leading to DNA fragmentation, thus indicating that DADS-induced apoptosis is caspase-3-dependent. BAPTA attenuated the $\Delta\psi m$ abrogation and significantly diminished the occurrence of DADS-induced apoptosis in HeLa cells. Activation of caspase-9 and caspase-3 indicated involvement of the intrinsic pathway of apoptosis. Results strongly suggested that the garlic compound DADS suppressed antiapoptotic factors and activated intrinsic caspase cascade for apoptosis in HeLa cells.

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PHENOTYPE SWITCHING, A NEW PARADIGM FOR METASTATIC PROGRESSION

Keith S. Hoek, Natalie C. Schlegel, Ossia M. Eichhoff, Daniel Widmer and Reinhard Dummer

Department of Dermatology, University Hospital of Zurich, 8091 Zurich, Switzerland

Metastatic melanoma is a complex and heterogeneous cancer for which, despite more than 100 clinical trials and over thirty years of dedicated molecular research, no effective therapy has yet been realised. Such failure demands we revisit the basic tenets which underly our assumptions about this disease and reappraise them in the light of recent findings. Transcription profiling of cell lines and short-term cultures by our group has uncovered among melanoma cells a previously unrecognized molecular taxonomy. *In vitro* and *in vivo* experiments show that this taxonomy is comprised of cells which are either programmed for proliferation, or programmed for invasion. Our interpretation of the data and subsequent experimentation has led us to conclude that the dominating paradigm for disease progression in melanoma, where weakly metastatic precursors evolve *via* the linear accumulation of changes into strongly metastatic cells, is in error. Instead, we hypothesize that once transformation has been achieved, melanoma cells express transcription programs (phenotypes) according to microenvironmentally derived signals, which in turn allows them to drive disease progression by switching between proliferative and invasive programs. This new phenotype-switching model answers several criticisms leveled at the present linear accumulation model, offers an explanation for how gene expression markers for early primary lesions frequently persist in metastases, and supplies a plausible reason for therapy escape.

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IMAGING THE TOTALITY OF CANCER PROGRESSION IN REAL TIME

Robert M. Hoffman

AntiCancer, Inc., San Diego, CA, USA

The development of GFP for imaging in the live animal has revolutionized *in vivo* biology, in particular, the study of metastasis (Nature Reviews Cancer 5, 796-806, 2005). The totality of cancer progression can now be followed in real time in the live animal, with many of the steps imageable non-invasively. GFP-expressing transgenic mice transplanted with the RFP-expressing cancer cells enable the distinction of cancer and host cells. This is particularly useful for imaging the tumor microenvironment, including tumor angiogenesis. Cancer–cell trafficking through the cardiovascular and lymphatic systems is the critical means of spread of cancer. The use of fluorescent proteins to differentially label cancer calls in the nucleus and cytoplasm and high-powered imaging technology are used to visualize the nuclear-cytoplasmic dynamics of cancer-cell trafficking and extravasation in both blood vessels and lymphatic vessels in the live animal. Proliferating and dormant cancer cells are readily distinguished in the live animal. This technology has furthered our understanding of the spread of cancer at the cellular and subcellular level in the live mouse. Fluorescent proteins thus enable both macro and micro imaging technology and thereby provide the basis for the new field of *in vivo* cell biology.

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***ETS-1* ONCOGENIC ACTIVITY IS MEDIATED *VIA* TGFα**

Chet E. Holterman, Aleksandra Franovic, Josianne Payette and Stephen Lee

Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario Canada K1H 8M5

Overt expression of the *Ets-1* oncogene has been demonstrated in a variety of tumors and has been correlated with poor patient prognosis and clinical outcome. While studies have established a role for Ets-1 in regulating the expression of genes involved in extracellular matrix remodeling and angiogenesis, relatively little is known regarding the mechanisms through which Ets-1 promotes primary tumor establishment. We previously elucidated a critical role for the TGFα/EGFR autocrine pathway in establishment of renal clear cell carcinoma (RCC).

Examination of the proximal *TGFα* promoter region revealed the presence of multiple Ets-1 binding sites. Through the use of reporter assays and quantitative PCR we demonstrate that the *TGFα* promoter is responsive to Ets-1 and that inhibiting Ets-1 activity *via* dominant negative Ets-1 or siRNA silencing of Ets-1 is sufficient to block both reporter activity and endogenous *TGFα* expression in 786-0 $VHL^{-/-}$ RCC cells. To confirm the physical interaction of Ets-1 with the *TGFα* promoter, we performed chromatin immunoprecipitation. *In vitro* silencing of Ets-1 blocked autonomous growth of 786-0 cells in a TGFα-dependent manner. Importantly, tumor xenograft assays demonstrate that silencing of Ets-1 prevents tumor formation in a manner that is rescuable by re-establishment of TGFα expression. We believe these results to be the first to clearly establish a role for Ets-1 in primary tumor formation through its ability to regulate TGFα expression.

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HORMONE-REFRACTORY PROSTATE CANCER-NEW OPTIONS FOR ITS MANAGEMENT

L. Holubec, J. Klecka, O. Topolcan, M. Pesta, V. Eret, J. Finek and M. Hora

Faculty Hospital and Medical School in Pilsen, E. Benese 13, 305 99 Plzen, Czech Republic

Prostate cancer is the most commonly diagnosed malignancy in men. Although many patients whose cancer has been diagnosed and treated at early stage can be cured, the yearly death toll of prostate is one of the leading causes of death among males. For the past 60 years, androgen deprivation has been the mainstay of treatment for advanced prostate cancer. Whether obtained by orchiectomy or by administration of an LHRH agonist, castration produces subjective and/or objective response in more than 80% of patients. Further androgen deprivation can still be obtained by combined therapy with an anti-androgen, in order to obtain "maximal androgen blockade". In spite of hormone therapy, all patients will progress and eventually become hormone-refractory. These patients retain hormonal sensitivity in that they may respond to secondary hormonal therapy. They belong to the androgen-independent and hormone-sensitive group according to the classification proposed by Scher. However "hormone refractory prostate cancer" (HRPC) is the term commonly used to describe prostate cancer that has progressed in spite of primary androgen deprivation or maximal androgen blockade. The standard of care of hormone-refractory prostate cancer is second-line hormone therapy which consists of antiandrogen withdrawal, corticosteroids, oestrogens or adrenal enzyme inhibitors. Second-line hormone therapy has poor activity which led to the use of chemotherapy. A number of cytotoxic agents have been tested against HRCP over the years. The aim of this overview lecture is to compare the efficacy of different chemotherapeutical regimens in patients with failure of the second-line hormonal supression and a probable hormonally independent cancer. Mitoxantrone plus prednisone reduces pain and improves the quality of life in men with advanced, hormone-refractory prostate cancer, but it does not improve survival. A number of clinical studies with a variety of protocols were initiated to evaluate the potential usefulness of Navelbine in HRPC. When used alone in a series of phase II clinical studies, Navelbine demonstrated its efficacy in terms of relief of bone pain, the major complaint of HRPC patients, and in terms of PSA response. Significant reductions of serum PSA levels, by 50% or more, were obtained in a number of patients. When used in combination with low dose of corticosteroids, as shown in a large phase III pivotal study, NAVELBINE appeared significantly more efficacious than hormone-therapy alone in terms of progression-free survival, PSA response and clinical benefit (pain intensity, analgesics consumption and performance status). When given with prednisone, treatment with docetaxel every three weeks led to superior survival and improved rates of response in terms of pain, serum PSA level, and quality of life, as compared with mitoxantrone plus prednisone. The literature overview and our personal experience from routine clinical practice provide evidence that cytotoxic chemotherapy can significantly prolong survival among men with hormone-refractory prostate cancer. The obtained data suggest that docetaxel plus prednisone is the preferred option for most patients with hormone-refractory prostate cancer. Also Navelbine appeard as a good candidate for clinical development in the chemotherapy of HRPC. In conclusion, chemotherapy open new perspectives in the treatment of HRPC.

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STEM CELL-LIKE SPHERE FORMING CELL POPULATIONS IN SARCOMAS. IMPLICATIONS IN CHEMORESISTANCE AND POSSIBLE THERAPEUTIC TARGETS

Kanya Honoki[1], Hiromasa Fujii[1], Yoshinori Takakura[1] and Toshifumi Tsujiuchi[2]

[1]Department of Orthopedic Surgery, Nara Medical University, 840 Shijo-cho, Kashihara 634-8521; [2]Department of Life Science, Faculty of Science and Technology, Kinki University, 3-4-1 Kowakae, Hiagshi-Osaka 577-8502, Japan

The presence of cancer stem cells, in both solid and hematopoietic malignancies, has been recently linked to their pathogenesis. Sarcomas are rare, and diversely characterized by degrees of mesenchymal differentiation. The aim of the current study was to demonstrate whether the sarcoma cell lines possess the stem-like properties, using human

osteosarcoma MG63, Ewing sarcoma HTB166, fibrosarcoma HT1080, and rat osteosarcoma COS1NR and malignant fibrous histiocytoma MFH1NR cell lines, both of which were induced by 4-hydroxy (amino) quinoline 1-oxide in F344 rats.

All cell lines possessed an ability to form spherical, clonal expanding colonies ('sarcospheres') in anchorage-independent, serum-starved conditions at the frequency of 0.2-0.8%. Sarcospheres showed stem-like properties with the ability of self-renewing, and expressed the stem cell-related genes including Nanog, Oct3/4 and STAT3. Rat sarcospheres showed strong tumorigenicity *in vivo via* their inoculation into syngeneic rats. Sarcospheres from human Ewing sarcoma and rat sarcoma cell lines remarkably reduced *INK4a-ARF* gene expression which is originally expressed in adherent cells. Finally, human sarcosphere cells showed chemoresistance to doxorubicin and cisplatin compared to adherent monolayered cells. These spheres showed increased expression of DNA repair enzyme genes, *MLH1* and *MSH2*, suggesting that the chemoresistance in stem-like sphere cells is partly due to the increased DNA repair ability after DNA damage induced by chemotherapeutic agents.

These results suggest that the stem-like cell populations exist in various kinds of sarcomas, and could be involved in chemoresistance, which leads to disease relapse, metastasis, and pathogenesis as well, and these may possibly provide novel targets in future sarcoma treatments.

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MEDICINAL ELECTRONOMICS BRICOLAGE: DESIGN OF HYPOXIA-TARGETING ANTINEOPLASTIC DRUGS

Hitoshi Hori[1], Yoshihiro Uto[1], Eiji Nakata[1] and Hideko Nagasawa[2]

[1]Department of Life System, Institute of Technology and Science, Graduate School, The University of Tokushima, Tokushima 770-8506;
[2]Laboratory of Pharmaceutical Chemistry, Gifu Pharmaceutical University, Mitahorahigashi-5, Gifu 502-8585, Japan

The hypoxic tumor microenvironment is now considered as a major factor that influences not only the response to antineoplastic therapies but also the potential for malignant progression and metastasis. We present here our progress in the development of hypoxia-targeting drugs, including antiangiogenic hypoxic cell radiosensitizers, sugar-hybrid hypoxic cell radiosensitizers and hypoxia-targeting ^{10}B delivery agents.

First, we designed chiral 2-nitroimidazole derivatives containing a 2-aminomethylene-4-cyclopentene-1,3-dione moiety as antiangiogenic hypoxic cell radiosensitizers. The 2-aminomethylene-4-cyclopentene-1,3-dione moiety is expected to show high electrophilicity, comparable to that of the 2-methylene-4-cyclopentene-1,3-dione moiety included in TX-1123 and tyrphostin AG17. Among the compounds tested, TX-2036 proved to be the strongest antiangiogenic hypoxic cell radiosensitizer by *in vitro* radiosensitizing assay, the chick embryo chorioallantoic membrane (CAM) assay, and protein tyrosine kinase (PTK) inhibition assay. Our results showed that these chiral 2-nitroimidazole derivatives having the 2-aminomethlene-4-cyclopentene-1,3-dione moiety as a potent antiangiogenic pharmacophoric descriptor might be promising lead candidates for the development of antiangiogenic hypoxic cell radiosensitizers.

As a second strategy, we designed sugar-hybrid hypoxic cell radiosensitizers targeting for enhanced tumor glycolysis, namely Warburg's effect, which was well characterized in hypoxic tumor cells. In all eight compounds, their LUMO coefficients were localized on the 2-nitroimidazole ring. Among these, fully acetylated-glucose containing hypoxic cell radiosensitizer TX-2244 was the most active radiosensitizer having both a higher *in vitro* radiosensitizing activity and lower hydrophobicity compared to misonidazole, known as a classical hypoxic cell radiosensitizer.

We also present here our development of an *in vivo* model using developing chick embryo to evaluate the radiosensitizing activity against solid tumor. Boron neutron capture therapy (BNCT) is a targeted radiation therapy that significantly increases the therapeutic ratio relative to conventional radiotherapeutic modalities and then the development of efficient tumor-selective ^{10}B delivery agents (boron-10 carriers) is of pivotal importance for the purpose. As a third strategy, we designed hypoxia-targeting boron-10 carrier hybrid conjugated a hypoxic cytotoxins such as tirapazamine or TX-402, or 2-nitroimidazole moiety to sodium borocaptate (BSH) molecule. Among the 2-nitroimidazole-BSH conjugates tested, TX-2060 maintained high ^{10}B concentrations in solid tumors more effectively than any other compound tested, and showed a significantly stronger *in vitro* radiosensitizing activity in the treatment of TX-2060 than that of BSH with neutron beam irradiation. Among these conjugates tested, TX-2100 had the most favorable characteristics for its sufficient concentration of ^{10}B in tumors and during irradiation. TX-2100 also showed significantly stronger *in vitro* radiosensitizing effect than BSH when irradiated with neutron beam. We suggest our hypoxia-targeting hybrid boron-10 carriers might be useful and promising boron-10 carrier candidates for BNCT.

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INFLUENCE OF STI571 (IMATINIB, GLIVEC) ON THE MIGRATORY BEHAVIOR OF HUMAM GLIOMAS *IN VITRO*

S. Horn[1], M. Möhlenbruch[1], M. Herr[1], A. Schollmayer[1], N. Hopf[2] and W. Wagner[1]

[1]Department of Neurosurgery, University of Mainz, Langenbeckstr.1, 55101 Mainz;
[2]Department of Neurosurgery, Katharinenhospital, Kriegsbergstr. 60, 70174 Stuttgart, Germany

Introduction: STI571 is a tyrosine kinase inhibitor, known to react toward Bcr-ABL, c-Kit and PDGF tyrosine kinase receptors (PDGF-R). STI571 acts by disruption of the ligand receptor autocrine loops for PDGF factor that are a pervasive feature of malignant astrocytoma. In this study we examined the influence of tyrosine kinase inhibitor STI571 on the migratory behavior of human glioma cells. *Methods:* Using RT-PCR, immunocytochemistry and flow-cytometry, we provided evidence of PDGF α/β receptor RNA presence and its cell surface expression in three human glioblastoma multiforme (GBM) cell lines and one fibrillary astrocytoma cell line. Migration rate (MR) was examined by the time-lapse individual cell migration assay (TIM-Assay), allowing continuous observation of cell features up to single cell level under defined conditions. STI571 was applied in concentrations corresponding to the drug level in cerebrospinal fluid and plasma *in vivo*. Migratory behavior was examined in a time-frame of 24 hours. One cell line was analyzed in a cycle conditioned treatment and a period of 8 days. TIM-Assay was performed at day 1, 3, 6 and 8. *Results:* All glioma cell lines showed PDGF α/β receptor expression. In most tested cell lines, MR decreased after treatment with STI571. The cells showed variable migratory characteristics. Cells of one glioblastoma multiforme showed an inherently low MR without STI571 treatment. In the other three cell lines migration decreased after incubation with the tyrosine kinase inhibitor, already at the lowest STI571 concentration. The cyclical treatment with STI571 resulted in a constantly reduction of the migratory behavior. *Conclusion:* STI571 affects migratory properties whereas PDGF α/β receptor expression could be relevant. Targeting of PDGF signaling pathway by tyrosine kinase inhibitors represents a promising strategy to interfere with the migration activity of glioma cells.

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EFFECTS OF PA-42 ON THE MOLECULAR MECHANISMS OF APOPTOSIS AND METASTASIS IN HUMAN ORAL SQUAMOUS CANCER CELLS (HSC-3)

Wen-Tsong Hsieh[1], Yi-Ting Chen[2], Hui-Yi Lin[3] and Jing-Gung Chung[4]

[1]Department of Pharmacology, [2]Graduate Institute of Basic Medical Science, [3]School of Pharmacy and [4]Department of Microbiology, China Medical University, Taichung, Taiwan, ROC

Squamous cell carcinoma of the oral cavity is the sixth most frequent cancer in the world. *Physalis angulata* is a traditional Chinese annual herb which has been reported to exhibit anticancer effect on several types of human cancer. In this study, we isolated the active compound PA-42 from *Physalis angulata* and found PA-42 caused cell cycle arrest in G_2/M phase, with decreasing cyclin A, cyclin B1 and cdc2 activity, but increasing wee1, p27 and p53 activity in HSC-3 cells. It induced DNA fragmentation, formation of DNA ladders in agarose gel electrophoresis and increased the levels of AIF, Bax, Bid, cleaved caspase-3, Fas, but reduced Bcl-2 in HSC-3 cells. In metastasis assay of cancer cells, PA-42 also inhibited migration by transwell migration assay and wound healing assay, and inhibited invasion by matrigel invasion assay. PA-42 reversed cytoskeleton from mesenchymal-like to epithelial-like, with increased E-cadherin (epithelial marker) and reduced α-smooth muscle actin (mesenchymal marker) in HSC-3 cells. PA-42 also caused a decrease in MMP-3 and GRB2.

From these results, we found that PA-42 arrests HSC-3 cells at the G_2/M phase by decreased several cyclins, but increasing p27 and p53 activity. It induced apoptosis *via* both extrinsic and intrinsic pathways in HSC-3 cells. It also suppressed metastasis including migration and invasion *via* reversed cell skeleton in HSC-3 cells. We conclude that PA-42 was able to inhibit cell proliferation, induce apoptosis and inhibit cell metastasis in HSC-3 cells.

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EXPRESSION OF CASPASE 14 IN HUMAN EPITHELIAL CANCER CELLS AND ITS POTENTIAL THERAPEUTIC EFFECTS

Rachel Dulebohn, Douglas Dickinson, Regina Messer, Mohammad Al-Shabrawy, Amany Tewfik, Kalu Ogbureke, Jill Lewis and Stephen Hsu

Department of Oral Biology, School of Dentistry, AD1443 Medical College of Georgia, Augusta, GA, 30912, USA

Background: Oral squamous cell carcinoma is a devastating malignancy with mortality rate consistently at 50%. Treatment of such oral cancer is often associated with disfigurement, which can have a traumatic impact patients. Exploration of novel approaches and innovative therapies is needed to combat oral cancer. We previously reported that *caspase-14*, a green tea polyphenol-activated gene that is expressed during terminal differentiation of certain epithelial cells, is able to induce cell death and reduce tumorigenicity in skin cancer cells A431 and salivary gland cancer cells HSG. However, the underlying mechanism is unknown and whether adenovirus-delivered

transient caspase-14 expression induces similar effects in human oral cancer cells is not known. To express exogenous human caspase 14 in oral squamous cell carcinoma cells (OSC2) by adenovirus vector, and determine the effects of caspase-14 expression on cell growth, cell death and tumorigenicity. *Methods:* The human oral cancer cell line OSC2 was infected by adenovirus-expressing GFP or caspase 14 cDNA. Expression of caspase 14 was confirmed by Western blotting. Cell morphology was monitored by microscopic photography, cell growth was measured by cell counting and BrdU assay, and cell viability was determined by MTT assay. In addition, the cancer cells were xenografted into athymic mice to determine the tumorigenicity. *Results:* Expression of caspase-14 induced an undefined cell death in OSC2 cells compared to the control cells. Cell growth and cell viability were inhibited significantly by caspase-14 expression. Xenograft of caspase-14-expressing OSC2 cells into athymic mice resulted in reduced tumorigenicity. This effect could be due to an inhibitory influence of caspase 14 on tumor vascularization. *Conclusion:* Oral cancer cells underwent growth inhibition and cell death when exogenous caspase-14 was expressed in these undifferentiated tumor cells. Caspase-14 expression in OSC2 cells also reduced tumorigenicity *in vivo*. Further effort is warranted to explore if caspase 14-expressing adenovirus could be used as a potential therapeutic approach to treat human epithelial cancer.

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ENHANCEMENT OF ANTITUMOR PROPERTIES OF TRAIL BY TARGETED DELIVERY TO THE TUMOR NEOVASCULATURE

Lin Cao, Pan Du, Shu-Han Jiang, Guang-Hui Jin, Qi-Lai Huang and Zi-Chun Hua

The State Key Laboratory of Pharmaceutical Biotechnology and Department of Biochemistry, College of Life Sciences, Nanjing University, 22 Hankou Road, Nanjing 210093, Jiangsu, P.R. China

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising anticancer agent with tumor-selective apoptotic activity. TRAIL plays a role in the innate and adaptive immune response, autoimmune disease, and may also be involved in hepatic cell death and inflammation. For these reasons, chronic exposure to TRAIL may have deleterious side-effects in patients as a cancer therapeutic. In this study, we have improved the antitumor activity of TRAIL by targeted delivery to the tumor vasculature, leading to dramatic enhancement of its therapeutic properties. TRAIL was fused to the ACDCRGDCFC peptide (named RGD-L-TRAIL), a ligand of $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins. Biological activity was evaluated *in vitro* and antitumor efficacy was investigated *in vivo* as a single agent and in combination with irinotecan hydrochloride (CPT-11). The fusion protein RGD-L-TRAIL, but not TRAIL or RGE-L-TRAIL, specifically bound to microvascular endothelial cells in a dose-dependent manner and showed enhanced apoptosis-inducing activity (caspase-3 and caspase-8 activation) in $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrin positive cancer cells. In addition, RGD-L-TRAIL was more effective in suppressing tumor growth of COLO-205 tumor-bearing mice than an equivalent dose of TRAIL. The antitumor effect of RGD-L-TRAIL was further enhanced by combination with CPT-11 in both TRAIL-sensitive COLO-205 and TRAIL-resistive HT-29 tumor xenograft models. Our findings suggest the novel fusion protein RGD-L-TRAIL can directly target tumor endothelial cells as well as $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrin-positive tumor cells. The tumor-targeted delivery of TRAIL derivatives, such as RGD-L-TRAIL, may prove to be a promising lead candidate for cancer therapy.

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KAEMPFEROL INDUCES APOPTOSIS *VIA* MITOCHONDRIAL- AND CASPASE-DEPENDENT CASPASE SIGNALING PATHWAYS IN HUMAN OSTEOSARCOMA U2OS CELLS

Wen-Wen Huang[1], Meng-Wei Lin[2], Kuei-Li Lin[3], Wai-Jane Ho[4], Chin-Fen Lin[5], Kun-Hong Li[6], Jai-sing Yang[7] and Jing-Gung Chung[1]

[1]School of Biological Science and Technology, [2]School of Pharmacy, [5]Department of Biochemistry, [6]School of Medicine and [7]Department of Microbiology, China Medical University, Taichung, Taiwan;
[3]Department of Radiation Oncology, Chimei Medical Center, Tainan, Taiwan;
[4]Department of Bioresources, Dayeh University, Changhua, Taiwan ROC

It is well known that flavonoids can block or suppress multistage carcinogenesis and also exert anti-neoplastic activites through the inhibition of cell growth and induction of apoptosis. Kaempferol is one of the flavonoids that usually exist in many plants. Recently, antioxidant activity has been used for cyto-protection. Kaempferol seems not only to prevent cells from free radical damage *via* antioxidant activity but also induce apoptosis *via* prooxidant activity in cancer cell lines. Kaempferol induced anti-proliferative activity in many transformed cancer cell types, such as rat H4IE cells, human HT29 colon cancer cells, acute leukemia cells, lung non-small carcinoma H460 cells. In this study, we examined the apoptotic effect and molecular mechanism of kaemperol in U2OS osteosarcoma cells. Kaempferol induced dose-dependent suppression of cell viability by MTT assay in U2OS cells. We used comet assay and DNA gel

electrophoresis to confirm kaempferol induced DNA damage and apoptosis. Flow cytometry was used to detect kaempferol-increased Ca^{+2} production and reduced levels of MMP. Kaempferol induced the release of cytochrome *c*, Apaf-1, AIF from mitochondria accompanied by activation of caspase-9, caspase-3 by Western blotting and caspase activity assay and real-time PCR. Specific inhibitors of caspases-9 and caspase-3 prevented caspase-9 and -3 activation and led to a decrease in the percentage of apoptosis. Kaempferol also promoted the expression of Bax, GRP78 and GADD153 but inhibited the expression of Bcl-2, Bcl-xL and IAP. Our results suggested that kaempferol is able to induce ER stress and apoptosis through the mitochondrial- and caspase-dependent signal pathways in U2OS cells.

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OUR CURRENT UNDERSTANDING OF THE ETIOLOGY OF COLORECTAL TUMORS: ONGOING RESEARCH FINDINGS FROM THE PLCO TRIAL

Wen-Yi Huang and Richard B. Hayes
for the PLCO research team

Division of Cancer Epidemiology and Genetic, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD20892, USA

Cancer of the colon and rectum (colorectal) ranks second in both incidence and mortality in developed countries, and the incidence is rising in developing countries. Adenomatous polyps are considered the key premalignant lesion leading to colorectal cancer, hence risk factors for adenoma are an important research topic.

We have carried out investigations of colorectal adenoma in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, including about 75,000 screened participants and 75,000 non-screened controls, at ten centers in the United States recruited between 1993 and 2001. Our studies related reduced adenoma risks to dietary fiber, dietary calcium, a gene variant in the calcium-sensing receptor, serum selenium, circulating vitamin D metabolites, serum insulin-like growth factor (IGF-1), use of non-steroid anti-inflammatory drugs (NSAIDs), and recent hormone replacement therapy. We related increased risks to carbohydrate intake and glycemic load, meat cooking practices, particularly PhIP exposure, and high body mass index. Tobacco use was strongly related to adenoma risk, with evidence of risk modification in relation to genes involved in the metabolism of tobacco smoke constituents, including *EPHX1, CYP1A1, NQO1, NAT1, NAT2,* and *GSTs*. We also characterized adenoma risk in relation to genetic variants in transforming growth factor beta 1 (*TGFB1*), nucleotide and base excision repair (NER, BER) genes, selected P53 response elements, alpha-methylacyl-CoA racemase (*AMACR*), the frizzled-related protein (*FRZB*), the hemochromatosis gene (*HFE*), and SNPs at chromosome 8q24 between 128.47 and 128.54. In ongoing work, we are expanding the questionnaire-based studies to include risk analysis for colorectal cancer, incident adenoma, and recurrent adenoma. We are expanding genotyping studies to include larger numbers of cases and controls and to deepen the pathway and gene region-specific SNP coverage. With recently acquired pathology samples, studies are planned to assess colorectal tumor risk in relation to tumor molecular sub-types and in relation to epigenetic and genome instability profiles.

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PROSTATE CANCER STEM CELLS

Elaine M. Hurt

National Cancer Institute-Frederick, Center for Cancer Research, Laboratory of Cancer Prevention, Cancer Stem Cells Section, Frederick, MD 21702, USA

Recently there have been several reports of cancer stem cells (CSCs) in hematological malignancies as well as in solid tumors, such as breast, colon, glioblastoma, and prostate cancer. CSCs are the tumor-initiating cells that give rise to the multiple cell types present within a heterogeneous tumor. Moreover, CSCs are resistant to many conventional chemotherapies, resulting in recurrence of the tumor. Therefore, the targeting of these cells is central to any therapy that is to be successful in eradicating cancer. However, successful targeting of these cells requires an understanding of the biology governing the CSC's unique properties of continued self-renewal, multipotent differentiation, and tumor initiation. Using prostate CSCs as a model, we are elucidating the molecular mechanisms underlying these properties.

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BIOLOGICAL INDICATORS OF PROGNOSIS IN HUMAN CANCER: AN EMERGING ROLE FOR LECTIN GALACTOSIDE-BINDING SOLUBLE 3-BINDING PROTEIN (LGALS3BP)

Stefano Iacobelli[1], Clara Natoli[1], Nicola Tinari[1], Maurizia[1], D'Egidio[1], Antonino Grassadonia[1], Alba Cumashi[1], Enza Piccolo[1], Diana Zambelli[2] and Katia Scotlandi[2], on behalf of the Consorzio Interuniversitario Nazionale per la Bio-Oncologica (CINBO)

[1]Department of Oncology and Neurosciences, University "G. d'Annunzio", Chieti-Pescara, Chieti;
[2]Laboratory of Oncologic Research, Istituto Ortopedico Rizzoli, Bologna, Italy

LGALS3BP, also known as Mac-2-binding protein or 90K, is a secreted glycoprotein that binds galectins, beta-1

integrins, collagens, and fibronectin, and has relevance in cell–cell and cell–extracellular matrix adhesion. Previous studies have shown that elevated tissue and serum expression levels of LGALS3BP correlate with a poor prognosis in several malignancies, including breast cancer, non-small cell lung cancer, prostate cancer and non-Hodgkin lymphoma. In a search for a molecular signature of potential prognostic value in Ewing's sarcoma (EWS), we identified and validated eight genes, representative of three different networks, in 56 EWS patients. High mRNA expression levels of *HINT1, IFITM2, LGALS3BP, STOML2* and *c-MYC* were associated with reduced risk of death and lower risk of developing metastasis. On multivariate analysis, LGALS3BP was the most important predictor of event-free and overall survival. The association between *LGALS3BP* mRNA and prognosis was confirmed at the protein level when expression of the molecule was determined in tumor tissues, but not in serum, indicating a role for the protein in the local tumor microenvironment. Engineered enhancement of LGALS3BP expression in EWS cells resulted in an inhibition of cell adhesion to basal lamina and reduction of cell migration and *in vivo* metastasis. Thus, we propose LGALS3BP as a novel, reliable cancer biomarker which may be associated with prognosis.

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INTERACTIONS BETWEEN NORMAL HUMAN FIBROBLASTS AND HUMAN PROSTATE CANCER (CaP) CELLS IN A COCULTURE SYSTEM

F. Iacopino, C. Angelucci, S. Ferracuti and G. Sica

Istituto di Istologia ed Embriologia, Facoltà di Medicina e Chirurgia, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Roma, Italia

Stroma affects the development of many organs and plays an important role in regulating epithelial malignancies, such as prostate cancer. Fibroblasts represent the major cell type of the stromal compartment. With the aim of clarifying the relationships between fibroblasts and epithelial cancer cells, we utilized a coculture system including CaP cell lines both androgen-sensitive (LNCaP) and -insensitive (PC-3, DU-145) and a human gingival fibroblast cell line (FG). Morphological aspects were analyzed under a phase contrast inverted microscope, proliferation in conditioned media (CM) was assessed by cell counts, and E-cadherin (E-cad) expression by immunocytochemistry. In the cocultures, androgen-sensitive LNCaP cells grew in a network on the top of the monolayer formed by the FG, while colonies of androgen-insensitive PC-3 and DU-145 cells were surrounded by FG. After six days, LNCaP cell number was apparently lower in the cocultures than in plates where they grew alone. Both cell lines underwent morphological changes. After six days, the growth of PC-3 and DU-145 overcame that of FG which were almost evanished. The CM of FG inhibited the proliferation of LNCaP cells, after three days by 33% ($p<0.01$) and after six days up to 82% ($p<0.01$), but had no effect on PC-3 and DU-145 cell growth. The CM of all three CaP lines reduced the growth of FG, being that of DU-145 cells the most effective (50% inhibition after three days, $p<0.01$, and 55% after six days, $p<0.01$). The FG did not express E-cad, while a strong staining was detected in LNCaP cells. PC-3 cells showed a nuclear staining while a sporadic membrane expression of E-cad was observed in DU-145 cells. In the cocultures, a reduction in the nuclear reactivity of PC-3 cells was found. The data obtained confirm the existence of a dialogue between FG and CaP cells, which results in both a peculiar modality of growth and a regulation of proliferation probably due to growth factors secreted in the culture medium.

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VERIFICATION BIAS OF OCCULT CANCER IN CASE–CONTROL STUDIES ON THE ASSOCIATION OF OXIDATIVE STRESS AND PROSTATE CANCER

Taro Iguchi[1,3], Ching Y. Wang[1], Nicolas B. Delongchamps[1,4], Gustavo de la Roza[2], Gabriel P. Haas[1] and Tatsuya Nakatani[3]

Department of [1]Urology and [2]Pathology, SUNY Upstate Medical University, Syracuse, NY, USA;
[3]Department of Urology, Osaka City University School of Medicine, Osaka, Japan;
[4]Department of Urology, University of Paris, Paris, France

Introduction and Objective: Epidemiological studies have correlated prostate cancer (PCa) with oxidative stress related gene polymorphism. Oxidative damage to nuclear DNA may result in carcinogenesis. Manganese superoxide dismutase (MnSOD) plays an important role in protection from reactive oxygen species-mediated DNA damage. Recent studies have suggested that *MnSOD* gene polymorphism is associated with PCa. However, some studies reported negative association. Many case–control studies on the molecular epidemiology of PCa do not take into consideration that the control group may include a significant proportion of occult PCa. In this study, we investigated the association of *MnSOD* genotypes and PCa in prostates obtained from deceased men, whose cancer status could be verified by complete histological evaluation. *Methods:* A total of 186 prostate glands from deceased men (age range 45-92 years) who had no known history of prostate cancer were eligible for analysis. An 18-core needle biopsy regimen and whole mount section of the prostate were performed. Genomic DNA was extracted from the normal portion of the prostate and *MnSOD* genotyping was

performed using PCR restriction fragment length polymorphism analysis. *Results:* When autopsied prostates were evaluated for cancer based on prostatic-specific antigen (PSA) <4 ng/ml, negative biopsy or both criteria, the incidence of PCa was 22%, 15% or 12%, respectively. The proportion of *MnSOD* AA genotype was significantly greater in the PCa than in non-PCa group. However, the inclusion of occult PCa in the non-PCa group reduced the odds ratio and increased the *p*-value. *Conclusion:* Because *MnSOD* AA genotype is associated with occult cancer, oxidative stress may result in the carcinogenesis of the prostate. However, this association may be masked in studies where the "cancer-free" control group contains cases of occult PCA. It is important to recognize that contamination of the control population by occult cancer reduces the reliability of the results. Rigorous characterization of the experimental and control groups is needed in order to preserve the integrity of the conclusions.

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IDENTIFICATION OF NOVEL GENES INVOLVED IN THE SYNERGISTIC ANTITUMOR EFFECT OF CAFFEINE IN OSTEOSARCOMA CELLS USING cDNA MACROARRAY

Sadao Ii[1], Yoshimichi Ueda[2], Miyako Shimazaki[2], Shogo Katsuta[2], Koutarou Takazawa[1], Yoshimitsu Kanazawa[1], Katsuro Tomita[1] and Hiroyuki Tsuchiya[1]

[1]Department of Orthopaedic Surgery, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa, Ishikawa 920-8641;
[2]Department of Pathology, Kanazawa Medical University, Ishikawa, Japan

Background: Caffeine enhances the cytocidal effects of DNA-damaging agents. This study investigated genes involved in the synergistic effect of caffeine on osteosarcoma cells using gene-profiling analysis. *Materials and Methods:* Sensitivity to cisplatin and the synergistic effect of caffeine were evaluated in five osteosarcoma cell lines. Gene expression profilings were analyzed using cDNA macroarray and verified by real-time RT-PCR. *Results:* The cell lines were grouped into three types with different cytotoxic patterns. Comparison of profiling data from these groups identified twelve novel genes associated with the synergistic effect of caffeine. Real-time RT-PCR analyses verified up-regulation of two apoptosis-enhancing genes and down-regulation of two interferon-inducible genes related to the synergy of caffeine. *Conclusion:* These findings provide new insights into the molecular mechanisms of the synergistic effect of caffeine in osteosarcoma, providing candidates for an assay of responsiveness to caffeine-potentiated chemotherapy for osteosarcoma.

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DEVELOPMENT OF A NEW 3-D CO-CULTURE SYSTEM FOR HUMAN BREAST CANCER PROGRESSION

S. Ashraf Imam

Molecular Pathology Program, Huntington Medical Research Institutes, Pasadena, CA, 91101, USA

Background: Patients with cancer receive therapies that tend to be directed at cancer cells, while sparing the surrounding non-cancerous cells which are collectively called stromal cells. One of the major types of stromal cells in tumor lesions, referred to as myofibroblast or cancer-associated fibroblast (CAF), is now recognized as a key factor influencing cancer progression. Moreover, it is also recognized that the present strategy of therapies of primary cancer are inadequate for eradication of every last viable cancer cell, hence the existence of residual viable cancer cells that are likely to cause treatment failure and recurrence. Yet, the CAF-derived contributing mediators of cancer progression have been overlooked as the potential targets for treatment of cancer patients. The progress in this newly recognized critical field of tumor microenvironment has been slow, mainly due to the technical difficulties of studying the significance of heterotypic cellular interaction in tumor progression in the presently available *in vitro* co-culture system. *Methods:* We recently developed a 3-dimensional microgravity co-culture system by overcoming the major difficulties of the presently available co-culture system. Using our newly developed system, breast cancer cell and breast CAF were successfully co-cultured. The co-cultured preparation, termed breast cancer histoid (BCH), was harvested, fixed in formalin, embedded in paraffin, sectioned at 4 μm-thickness and immunohistochemically (IHC) stained. *Results:* The sections of BCH showed invasion of the core CAF by the cancer cells which were positive for the expression of cytokeratins and C-erbB-2, where as the CAFs were negative. Conversely, CAFs exhibited the expression of α-smooth muscle actin, whereas the cancer cells were negative. The stroma was positive for the expression of collagen IV. *Conclusion:* The system facilitated generation of BCH in a simulated tissue-like microenvironment, thereby allowing heterotypic interaction, invasion of CAF core by the cancer cells and their growth in the endogeneously produced extracellular matrix. The BCH has a potential to be used as a basis for the identification of molecular mediators of breast cancer progression which could be therapeutically targeted in not only the cancer cells but also the stromal cells, as a new approach to therapy of patients with primary breast cancer.

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UNMETHYLATED *E-CADHERIN* GENE AND OVEREXPRESSION OF E-CADHERIN PROTEIN ARE ASSOCIATED WITH METASTATIC HUMAN PROSTATE CANCER CELLS IN BONE

B. Saha[1], P. Kaur[1], D. Tsao-Wei[2], W.Y. Naritoku[3], S. Groshen[2], R.H. Datar[3], L.W. Jones[1] and S.A. Imam[1]

[1]Molecular Pathology Program, Huntington Medical Research Insts., Pasadena, CA, 91101; [2]Departments of Preventive Medicine, and 3Pathology, USC Keck School of Medicine, Los Angeles, USA

Background: The hypermethylation of *E-cadherin* gene and a reduction and/or loss of E-cadherin protein expression have been shown to be associated with the poorly differentiated primary prostate cancer cells. However, the status of their expression remains elusive in metastatic cancer cells in bone, the most prevalent site for metastatic growth. *Aim:* The study was undertaken to ascertain the methylation status of *E-cadherin* gene, a most frequent and known epigenetic mechanism of its regulation, and E-cadherin protein expression in biopsy prostate tissue specimens. Methods: The methylation status of *E-cadherin* gene was determined by methylation specific-PCR and E-cadherin protein expression by immunohistochemical staining method in confirmed cases of benign prostate hyperplasia (BPH) (n=11), primary prostate carcinoma (n=20) or metastasis to bone (n=15). *Results:* The BPH cells expressed unmethylated *E-cadherin* gene and homogeneous membraneous expression of E-cadherin protein in 10 out of 11 (91%) cases, whereas the methylated gene and heterogeneous protein expression of the protein were concurrently detected in the remaining case. The primary prostate cancer cells exhibited methylated gene and heterogeneous expression of the protein in 14 out of 20 (70%), where as the unmethylated gene and heterogeneous expression of the protein was detected in 3 out of 20 (15%) of cases. As compared to the BPH cells, a statistically significant increase in methylation of *E-cadherin* gene and reduction of E-cadherin protein expression was detected in the primary prostate cancer cells ($p>0.001$). In contrast to the primary cancer, a significantly increased frequency of metastatic prostate cancer cells in bone exhibited unmethylated *E-cadherin* gene and homogeneous expression of E-cadherin protein in 13 out of 15 (87%) of cases, and unmethylated gene with heterogeneous expression of the protein in the remaining 2 cases (McNemar $p<0.001$). *Conclusion:* This new observation demonstrated that the unmethylated *E-cadherin* gene and E-cadherin protein expression are significantly associated with distant metastatic prostate cancer cells in bone and that the high frequency of its expression may have a intercellular adhesion function in the formation of lesions of metastatic cancer cells in bone.

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HPV 16-ASSOCIATED TUMOURS: *IN VITRO* RESPONSES OF IMMUNE MICE AND MICE CURED WITH CHEMOTHERAPY AND SUBSEQUENT IL-12 IMMUNOTHERAPY

Marie Indrová, Jana Bieblová, Jan Bubeník and Milan Reiniš

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, v. v. i., Vídeňská 1083, Prague 4, Czech Republic

We have used an animal model for HPV16-associated tumours with distinct levels of MHC class I expression, TC-1 (MHC class I^+) and TC-1/A9 (MHC class I^-) to examine the immune responses and production of cytokines after immunotherapy with irradiated cellular vaccine producing IL-12 (TC-1-IL-12) of minimal residual tumour disease induced by ifosfamide derivative CBM-4A. Additionally, we assessed the immune responses and production of cytokines after immunization of healthy mice with irradiated TC-1 or TC-1/A9 tumour cells.

The expression of MHC class I molecules on tumour cells used for vaccination had no effect on the Th1/Th2 polarization in the spleen of mice after chemotherapy and subsequent immunotherapy, nor in the spleens of mice after immunization with two doses of irradiated TC-1 or TC-1/A9 cells. However, in both protocols, up-regulation of cytokine production was detected and compared to control animals. Chemotherapy and subsequent IL-12 immunotherapy of MHC class I-positive TC-1 tumours with the TC-1-IL-12 vaccine, as well as immunization with irradiated tumour cells, led to significant up-regulation of Th1 cell-produced cytokines IL-2 and IFNγ by the spleen cells. Significant up-regulation of IL-5, but not of IL-4 (Th2 cytokines) was also observed

In the ^{51}Cr microcytotoxicity assay, cytotoxic $CD8^+$ cells were found in the spleens of TC-1 (MHC class I^+) but not of TC-1/A9 (MHC class I^-)-tumour-bearing mice treated by combined chemo-immunotherapy with CBM-4A and cellular vaccine producing IL-12, as well as in the spleens of mice immunized with irradiated tumour cells. In the spleens of TC-1/A9 but not of TC-1 tumour-treated animals, NK activity measured as the lysis of NK-sensitive YAC-1 targets was detected.

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CELLULAR BIOLOGY OF BREAST CANCER, FOCUSING ON GENOMIC AND CLINICOPATHOLOGICAL EVENTS IN CARRIERS OF A *BRCA2* FOUNDER MUTATION

Sigurdur Ingvarsson

Institute for Experimental Pathology, University of Iceland at Keldur, v/Vesturlandsveg, 112 Reykjavik, Iceland

Like other cancer types, breast cancer is considered to be a genetic disease. While the majority of genetic changes are somatic, a minority are in germline. About 10-20% of breast cancer is thought to be due to a germline mutation in high-penetrance genes, where the major focus has been on *BRCA1* and *BRCA2*. Hereditary differs from sporadic breast cancer with respect to phenotype, chromosomal gains and losses, somatic mutations and expression pattern. TP53 pathways are altered in carriers of *BRCA1* and *BRCA2* mutations, and *BRCA1* carcinomas frequently carry *TP53* mutations. There is strong evidence that genomic instability has a role in breast cancer pathogenesis, particularly in hereditary breast cancer, and possibly a role in sensitivity and resistance to therapy. Some germline mutations are defined as founder mutations. Founder mutations permit analysis of a large number of cases, and provide more accurate information on penetrance, expression, and genetic and environmental modifiers of risk. Population studies are also valuable due to the possibilities for evaluating clinicopathological data in a group of patients who have the same mutation. In Iceland a rare founder mutation has been detected in *BRCA1*, and a frequent one in BRCA2. In addition to population-based studies on genetics and clinicopathology, an extensive analysis of somatic changes in tumours of *BRCA2* founder mutation carriers has been made. As the Icelandic carriers of *BRCA* mutations all have the same alteration, this can be considered an ideal model population to analyze the influences of somatic mutations acquired during carcinogenesis and tumor progression and effects of low-penetrance modifier genes. This information can be useful in understanding the role played by these genes in the incidence of breast cancer, in order to target genetic testing, provide individual risk assessment, and design better therapeutic strategies. The evidence of differences in susceptibility and in age of onset among carriers of a specific mutation makes it easier to define the role and importance of risk-modifying factors, leading to improved disease management.

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BEE VENOM-INDUCED G_0/G_1 PHASE ARREST AND APOPTOSIS IN HUMAN BLADDER CANCER TSGH-8301 CELLS *VIA* REACTIVE OXYGEN SPECIES AND MITOCHONDRIA-DEPENDENT PATHWAY

Siu-Wan Ip[1], Yung-Lin Chu[1] and Jing-Gung Chung[2]

Departments of [1]Nutrition and [2]Biological Science and Technology, China Medical University, No.91 Hsueh-Shih Road, Taichung, Taiwan 40402, ROC

Bee venom (BV) has been shown present biological activities including inhibit growth and induce apoptosis in human cancer cells. However, there are no reports to address the molecular mechanisms involved in BV-induced apoptosis in human bladder cancer TSGH-8301 cells. In this study, we showed that BV reduced the percentage of viable TSGH-8301 cells and the IC_{50} is 10 μg/ml in the BV-induced cell death. Flow cytometer also demonstrated that BV induced G_0/G_1 arrest, increased the levels of reactive oxygen species (ROS) and Ca^{2+} production and reduced the change in mitochondrial membrane potential ($\Delta\Psi m$) in TSGH-8301 cells. BV-induced apoptosis was also confirmed by 4,6-diamidino-2-phenylindole (DAPI) staining, Comet assay and DNA gel electrophoresis. The results indicated BV induced cytotoxicity and apoptosis in dose-dependent manners. It is suggested that BV inhibits the proliferation of human bladder cancer TSGH-8301 cells and induced apoptosis through induction of ROS. Consequently, BV can be used for clinical treatment in bladder cancer patients in future.

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EGFR AMPLIFICATIONS AND MUTATIONS IN LUNG CANCER

Sofi Isaksson[1], Annette Salomonsson[1], Pär-Ola Bendahl[1], Leif Johansson[2], Per Jönsson[3], Mats Jönsson[1], Göran Jönsson[1] and Maria Planck[1]

Departments of [1]Oncology, [2]Pathology and [3]Thoracic Surgery at Lund University Hospital, Sweden

Epidermal growth factor receptor (EGFR) is one of four members of the HER family of cell surface receptors. These are involved in the intracellular signaling which results in altered gene expression and stimulation of cell proliferation. EGFR is expressed or overexpressed in a wide variety of solid human tumors, including lung cancer of the non-small cell type (NSCLC), which constitutes eighty percent of all lung cancer. Some patients with advanced NSCLC may benefit from treatment with tyrosine kinase inhibitors (TKIs) directed against EGFR. EGFR protein expression, *EGFR* gene copy numbers, *EGFR* mutation status, and alterations in molecules acting downstream of EGFR, have all been suggested to be important in predicting response to these drugs, but no consensus regarding the preferred choice of predictive biomarkers has yet been achieved.

We analyzed the spectra of *EGFR* mutations and amplifications in 62 freshly frozen biopsies of early-stage primary NSCLC tumors (48 adenocarcinomas and 14 squamous cell carcinomas) using quantitative PCR of *EGFR* and direct sequencing of exons 18-21. Furthermore, we studied the genetic profiles of the tumors in relation to EGFR status by array-based comparative genomic hybridization (aCGH) using whole-genome tiling resolution bacterial artificial chromosome (BAC) microarrays.

Amplification of the *EGFR* gene (qPCR ratio ≥1.5) was demonstrated in 3/48 adenocarcinomas and in 4/14

squamous cell carcinomas. Mutation (substitution, insertion or deletion) was found in 7 adenocarcinomas. Two of these harbored both mutation and amplification. Seven *EGFR* amplifications (at a level corresponding to at least 4 gene copies) were detected by aCGH, and all of these could be confirmed by qPCR, and *vice versa*. The gene copy number profiles of the *EGFR*-altered tumors, as revealed by aCGH, were complex and harbored frequent alterations in regions containing known or presumed oncogenes and tumor suppressor genes.

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DETAILED PHARMACOLOGICAL EVALUATION OF A TUMOR-INHIBITING RUTHENIUM (III) COMPLEX WITH DICHLORO-BIS(ETHYL 2-AMINO-4-PHENYL-5-THIAZOLE- CARBOXYLATE) RUTHENIUM(III) CHLORIDE, CYTO-TOXICITY, INDUCTION OF APOPTOSIS, DNA-BINDING AND IN VIVO ANTINEOPLASTIC EFFICACY

Georgi Momekov[1], Iva Ugrinov[1,2], Evdokia Pashev[1,2], Spiro Konstantrinov[1,2], Dilyan Ferdinandov[1], Antonina Nikolov[1,3] and Darvin Ivanov[3]

[1]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University-Sofia, 2 Dunav Str., 1000 Sofia;
[2]Institute of Molecular Biology, Bulgarian Academy of Sciences, Acad. G.Bonchev Str, bl. 21, 1113 Sofia;
[3]Department of Chemistry, Faculty of Pharmacy, Medical University-Sofia, 2 Dunav Str., 1000 Sofia, Bulgaria;
[4]Unit of Toxicology and Chemotherapy, German Cancer Research Center, 69 120 Heidelberg, Germany

A detailed pharmacological evaluation of a novel ruthenium (III) coordination compound with dichloro-bis(ethyl 2-amino-4-phenyl-5-thiazolecarboxylate) ruthenium (III) chloride (**L**) was carried out. The cytotoxic activity of **L** was tested using the MTT-dye reduction assay against a broad spectrum of human tumor cell lines. The tested ruthenium complex exhibited significant concentration-dependent cytotoxic effects comparable or even superior to that of cisplatin. As evidenced by the observed oligonucleosomal fragmentation of genomic DNA following treatment of tumor cells with **L** its cytotoxic effects are at least partly mediated by induction of apoptosis. The DNA binding of **L** and cisplatin were assessed using a 40 n.b. fragment (5' CGCTATCGCTACCTATT-**GG**-ATCCT TATGCGT TAGTGTATG 3'), whereby the present GG-motif is the recognition sequence of the nuclease Bam H1. The level of DNA modification was determined after Bam H1 treatment, 5% polyacrilamide gel electrophoresis and ethidium bromide staining. Cisplatin completely inhibited the BamH1-mediated fragmentation of the target DNA-molecule indicating high platination capacity. The complex also significantly inhibited the fragmentation of the target DNA sequence. **L** exerted significant antineoplastic activity against the murine transplantable tumor Lewis lung carcinoma as evidenced by the observed increase of the life span (ILS) in the treated vs. untreated animals.

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SYNTHESIS AND CYTOTOXIC ACTIVITY OF 3-METHOXY-SALICYLALDEHYDE BENZOYLHYDRAZONE

Boriana Nikolova-Mladenova[1], Georgi Momekov[2] and Darvin Ivanov[1]

[1]Department of Chemistry, Faculty of Pharmacy, Medical University-Sofia, 2 Dunav Str., 1000 Sofia;
[2]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University-Sofia, 2 Dunav Str., 1000 Sofia, Bulgaria

A new chelating agent 3-methoxy-salicylaldehyde benzoyl - hydrazone (m-SBH) was synthesized. The ligand was prepared by the Schiff base condensation between benzoylhidrazine and 3-methoxy-salicylaldehyde in ethanol. The compound was characterized by elemental analysis, spectroscopic methods (IR, NMR) and thermal analysis. The new compound was investigated for cytotoxicity using MTT-dye reduction assay on several human cell lines. m-SBH showed a concentration-dependent inhibitory activity on SKW-3, HL-60, BV-173 and K-562 cell lines. The preliminary mechanistic investigations showed that the cytotoxicity of the compound is mediated by induction of apoptosis.

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RADIOEMBOLIZATION USING ^{90}YTTRIUM MICROSPHERES IN PRIMARY AND METASTATIC LIVER CANCER

Tobias F. Jakobs[1], Ralf-T. Hoffmann[1], Klaus Tatsch[2] and Maximilian F. Reiser[1]

Departments of [1]Clinical Radiology, and [2]Nuclear Medicine, University of Munich, Grosshadern Campus, Marchioninistrasse 15, 81377 Munich, Germany

Most patients with colorectal, breast, lung, hepatocellular and pancreatic cancer are at risk for recurrence from metastatic disease in the liver after potentially curative treatment. When the liver harbors metastatic or primary cancer, treatment options are reduced and patient survival rates rapidly decline. Unfortunately, uncontrolled liver disease is the most common final pathway for nearly half of all patients with colorectal cancer and the majority of people diagnosed with hepatocellular cancer. It is a worldwide health issue and a leading cause of premature death.

Anticancer Treatment Moves Forward with Microspheres: Today, there is new hope in the push to eradicate cancer from the liver through use of cutting-edge imaging technology to assist in delivery of microscopic radioactive spheres. These microspheres go directly to the tumor site, attacking cancerous cells while sparing healthy tissue. A multimodality approach, which utilizes the skills and expertise of several specialties – including diagnostic radiology, interventional radiology, radiation oncology and nuclear medicine – enables the radiation to be safely administered directly into the liver tumors. There are currently two forms of microspheres: SIR-Spheres® microspheres and Nordion's TheraSpheres. SIR-Spheres, which are polymer-based and manufactured by Sirtex, are currently the only FDA-approved microspheres for treatment of metastatic liver cancer. Nordion's TheraSpheres are made of glass beads that are irradiated. Though they are not FDA-approved, TheraSpheres have received "humanitarian device use" in the United States for hepatocellular carcinoma (HCC). Due to its FDA-approved status, the data presented in this presentation predominantly refer to SIR-Spheres. Physicians are encouraged by the early and impressive results of microspheres therapy, and patients often cite the minimal side-effects and ease of treatment.

Microspheres Background: Each SIR-Spheres microsphere is composed of resin and yttrium-90, a pure beta emitter that only penetrates tissues up to 11 mm. The radioactive source ^{90}Y does not come off of the microspheres, so by restricting the spheres to the tumor, the radiation is also localized exclusively to that area. Therefore, when microspheres are in the tumor – where they remain permanently – they will destroy only cancerous cells, and spare the surrounding normal liver tissue. The infusion of microspheres is performed in the angio suite primarily by an interventional radiologist who has experience with cheomoembolization. A key feature of delivering microspheres to the tumor is the fact that 80 to 100 percent of the blood supply to metastases in the liver comes from the hepatic artery. The latest in microcatheter and diagnostic interventional radiology C-arm technology allows for precise placement of radiation into small, peripheral vessels. During the procedure, the catheter is positioned in the liver with x-ray guidance by an interventional radiologist to allow for targeted infusion of the SIR-Spheres to the liver tumors. Since the treatment has to be precise, the role of imaging cannot be underestimated. The amount of radiation delivered can be significant, but because the microspheres are only about 32 microns in diameter, they enter the tumor from the hepatic artery supply and preferentially collect in the periphery of tumor nodules. The capillary beds will only allow passage of particles that are less than 10 microns, thereby trapping the microspheres within the tumor.

Imaging Guiding during SIR-Spheres Microspheres Infusion. Imaging is critical in all phases of internal radiation therapy. The treatment process starts with establishing the location, size and volume of tumors in the liver. At the same time, there also is a search for any extrahepatic tumor deposits. Using a PET-CT System, complementary information is obtained on the location and cellular activity of tumor deposits in three dimensions. High-resolution three-phase liver imaging is obtained with CT, followed by hepatic arterial system mapping internally with angiography. Next, the hepatic vasculature and potential release point of the microspheres are tested with a macro-aggregated albumin scan (^{99m}TcMAA), which uses the ubiquitous human serum protein albumin, processed into the same size as the radioactive microspheres, but bound to a gamma-emitting isotope for imaging instead of a beta source that is used for therapy, which cannot be easily imaged. The albumin particles lodge in the tumor just like the microspheres do, and thus a 'simulation' of the treatment is performed to detect deposition of particles in the stomach, small bowel or lungs instead of the intended target, the liver tumors. Corrective measures can then be taken to make microsphere delivery safer by avoiding extrahepatic spread.

Results: In this presentation, we review the results of ^{90}Y radioembolization in patients with non-resectable, otherwise non-responding, primary or secondary liver cancer.

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APPLICATION OF A TRANSMISSION LOW-COHERENCE DIGITAL HOLOGRAPHIC MICROSCOPE IN CANCER CELL BIOLOGY

Hana Janeckova[1], Pavel Vesely[2] and Radim Chmelik[1]

[1]Institute of Physical Engineering, Faculty of Mechanical Engineering, BUT, Technicka 2, Brno, 616 69;
[2]Institute of Molecular Genetics, AS CR, Flemingovo namesti 2, Prague 6, 166 37, Czech Republic

Initial experiments with the transmission digital holographic microscope (DHM) proved that the system is a usable instrument for imaging of cells, especially for monitoring of fast responses of living cells to external stimuli. Here we decided to exploit two distinguished properties of the DHM imaging in an *in vitro* study of cancer cell behaviour.

The first application was imaging of living cells attached to a cover slip at the bottom of the imaging chamber and immersed in an opalescent medium. To simulate these conditions we made a standard culture medium turbid by adding of polystyrene micro spheres (with a diameter of 356 nm). So we obtained a strongly scattering medium which filled the whole chamber and covered the cells with a 0.8 mm thick diffusing layer. The simulation showed that due to the capability of the microscope to shorten the coherence length of the illumination, the DHM can offer information about the condition of cells *in situ*ations where standard microscopy is failing.

The second application used a computed 3D presentation of cellular activity that is deemed to be more informative about fast cellular reactions that involve sudden and transient changes of the cell height and the distribution of the cell mass. Indeed in an experimental series designed to study the resistance and the type of reaction of various cancer cells to acute nutritional and energy deprivation we found this type of visualization advantageous (videos will be demonstrated). We obtained similar experience with comparative imaging of apoptosis induced by cisplatinum treatment. The assessment of DHM imaging of cultured cells performed so far was done in stationary single-use chambers only. Employment of a through-flow chamber (under development) is expected to be even more instrumental.

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RELEASE OF CFSE FROM NSCLC CELLS CULTURED WITH DIFFERENT TYPE OF DC "VACCINES" AND INFLUENCE OF TUMOUR LYSATE ON STATE OF LYMPHOCYTE ACTIVATION

Olga Jankowska[1], Paweł Krawczyk[1], Dariusz Sagan[2], Kamila Wojas-Krawczyk[1], Jacek Roliński[3] and Janusz Milanowski[1]

[1]Department of Pneumonology, Oncology and Allergology Medical University of Lublin;
[2]Department of Thoracic Surgery Medical University of Lublin;
[3]Department of Clinical Immunology, Medical University of Lublin, Poland

Immunotherapy is one of the most promising methods of cancer treatment. Vaccines against cancer aim to induce tumour-specific effector T-cells that can change the immunological system and reduce tumour mass. At present, there is no standardized methodology for preparing vaccines, and many questions concerning the optimal source and type of antigens as well as DC maturation state and activity still need to be answered.

The aim of our study was to investigate *in vitro* efficiency of autologous DCs to destroy NSCLC cells, the state of lymphocyte activation and ability of lymphocytes for cytotoxic reaction in two different types of DC stimuli.

After surgical resection, small tumour fragments were placed in culture dishes containing different growth factors. Seven days after operation, peripheral blood mononuclear cells (PBMCs) were cultured in RPMI-1640 medium supplemented with 10% autologous plasma in the presence of rhIL-4 and rhGM-CSF to generate immature autologous DCs. Mature DCs were obtained after incubation with TNF-α and tumour cell lysate or only with TNF-α. Cultures consisted of DCs, lymphocytes and macrophages. Cancer cells stained with CFSE were co-cultured with different kind of DCs. Flow cytometry, confocal microscopy and fluorometry were used in the investigation.

In this study, we found a significant increase in CFSE fluorescence level in supernatant from culture with tumour lysate and without tumour lysate in comparison with the pure tumour cell culture (p=0.03 and p=0.002, respectively). There was slightly higher CFSE fluorescence in supernatants from culture without tumour antigens in comparison with tumour lysate culture. We discovered a significantly positively correlation between expression of CD83 antigen on DCs and CFSE fluorescence level in culture without tumour lysate. We also observed a significantly negative correlation between the expression of CD83 antigen and percentage of CD83+ cells in the same culture.

In our study, we discovered a significantly higher percentage of cells producing IFN-γ in only TNF-α cultures in comparison with tumour cell lysate cultures (10.91±6.53% *versus* 8.95±4.86%; p=0.01). We also found a statistically significant positive correlation between percentages of CD69+ lymphocytes and percentage of cells producing IFN-γ in only TNF-α cultures (R=0.61; p=0.01) and (R=0.74; p=0.002) in culture with tumour cell lysate.

The present study suggests that autologous DCs stimulated solely by TNF-α were fully competent and may phagocytize cancer cells. It seems possible that DCs loaded with tumour antigens become too mature and lose phagocytolytic qualities. Such a vaccine may serve as a novel immunotherapy method in lung cancer treatment. Our research showed that mature DCs have the ability for cancer antigen presentation and could be able to activate cytotoxic T lymphocytes in both types of cultures. The significantly lower percentages of CD69+ lymphocytes and cells producing IFN-γ in cultures with tumour cell lysate confirmed the reduction (depletion) of DC function. This result suggests that tumour lysate may contain different compounds, responsible for cell inhibition.

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ROLE OF LOW PENETRANCE GENES IN FAMILIAL BREAST CANCER SUSCEPTIBILITY

Lilian Jara

Human Genetics Program, Institute of Biomedical Sciences, School of Medicine, University of Chile, Chile

Breast cancer (BC) is the most common cancer in women in the world. It has been estimated that one out of every nine women will develop BC during their lives. Hereditary BC

accounts for around 5-10% of all BC cases. Germline mutations in the two major susceptibility genes *BRCA1* and *BRCA2* (*BRCA1/2*) account for aproximately 20% of the familial BC cases. Thus, it was possible to infer the potential involvement of additional susceptibility genes that would account for low to moderate breast cancer risk. This is in accordance with the most widely accepted model which proposes that familial BC susceptibility is a consequence of a small number of mutations in *BRCA1/2* and a much higher proportion of mutations in ethnic-specific genes of moderate and/or low penetrance.

Several studies have reported that mutations in genes involved in DNA repair and in the maintenance of genome integrity may be responsible in the increase of cancer risk, including *RAD51, CHEK2, XRCC3* and *ATM*.

Human *RAD51* is known to function in DNA repair and interacts with a number of proteins implicated in BC, such as *BRCA1/2*. Using a case–control design, we have studied the *RAD51* 135G>C polymorphism (c.-98G>C, rs1801320) to evaluate its possible association with BC susceptibility. Our results showed an association of *RAD51* 135C genotypes (G/C and C/C) with an increased BC risk only among women with: a) a family history of BC, b) *BRCA1/2*-negative tumors and c) with age of onset <50 years (p=0.020, OR=2.17 [95%CI 1.11-4.24]). Therefore, our results allow us to propose that RAD51 135G>C polymorphism increases the risk of familial BC in women, with age of diagnosis <50 years, and this polymorphism may be a BC risk variant.

With respect to *CHEK2,* we analyzed the 1100delC mutation in 1320 individuals (196 breast cancer patients, 500 healthy Chilean females, and 624 healthy Chilean females but with at least two first or second degree relatives with BC). None of the analyzed samples carried the *CHEK2* 1100delC mutation. This finding suggests that this mutation is not either present or is present at an extremely low frequency in Chilean families with familial BC. Therefore, this variant has no practical importance for the clinicians in our population. We also raise the hypothesis that the 1100delC mutation is not present in some of those contemporary South American populations that stem from the admixture of Amerindian peoples with the Spanish settlers.

The *ATM* gene has been frequently implicated in hereditary BC as a low-penetrance susceptibility gene. The ATM kinase has an essential role in maintaining genomic integrity. We carried out a full mutation analysis of the *ATM* gene. We detected two missense variants and eight intronic polymorphisms. We also perform a case–control study between a subgroup of *BRCA1/2*-negative cases and controls for the 5557G>A missense variant and the IVS38-8T>C and the IVS24-9delT polymorphisms. Carriers of the IVS24-9delT/IVS38-8T>C/5557G>A composite triheterozygous genotype showed an increase in BC risk (OR=3.09 [95%CI 1.11-8.59], p=0.024). This composite genotype alone, or in combination with certain genetic background and/or environmental factors, could modify cancer risk by increasing genetic instability or by altering the effect of the normal DNA damage response.

Our results show that genetic tests are relevant in defining individual risks for hereditary BC.

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THE COMBI-TARGETING CONCEPT: ENGINEERING ANTICANCER MOLECULES TO BLOCK MULTIPLE INTRACELLULAR TARGETS

Bertrand J. Jean-Claude, Qiu Quyi, Zakaria Rachid, Fouad Brahimi, Anne-Laure Larroque, Sherin Al-Safadi and Juozas Domarkas

McGill University (MUHC), Royal Victoria Hospital, Montreal, Quebec, Canada

Overexpression of the epidermal growth factor receptor (EGFR) and its closest homologue HER2 have been associated with aggressive tumour progression and reduced sensitivity to DNA-damaging agents. In order to block the progression of refractory tumours overexpressing EGFR, we developed a novel strategy termed “combi-targeting” that sought to design molecules capable of not only blocking EGFR tyrosine kinase but also damaging DNA. These molecules, termed combi-molecules (CMs), in addition to being EGFR inhibitors on their own, were shown to release under physiological conditions another inhibitor of EGFR, and to be potent against EGFR-expressing tumour cells of various origins. These include breast, prostate carcinoma of the vulva and brain tumour cells. However, despite their binary targeting mechanism, their growth inhibitory IC_{50} values were still in the high micromolar range. In order to augment the potency of the CMs, we re-designed them to not only release a quinazoline inhibitor of EGFR, but also bi-functional alkylating moieties prone to induce DNA cross-links. More importantly, molecules termed “double-armed combi-molecules” were further designed to contain two quinazoline moieties and a central *N,N*-bis(2-aminoethyl)methylamine spacer which, following degradation, yielded higher concentrations of free inhibitors + cytotoxic bifunctional DNA damaging species. These molecules (*e.g.* ZRBA4, JDE52) were proven to be more effective than their predecessors and corresponding combinations of classical alkylators+inhibitors of EGFR. In this presentation, the potency of such type of novel combi-molecules against EGFR-overexpressing human tumour cells and their interactions with EGFR-mediated cell signaling will be analyzed.

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ALTERATION OF HEPATIC STELLATE CELLS IN ASCORBIC ACID DEFICIENCY AND AGING

Il-Hwa Hong[1], Jung-Youn Han[1], Jin-Kyu Park[1], Moon-Jung Goo[1], Ok-Kyung Hwang[1], Kyung-Sook Hong[1], Ae-Ri Ji[1], Mi-Ran Ki[1], Sung-Yong Hwa[2] and Kyu-Shik Jeong[1]

[1]Department of Pathology, College of Veterinary Medicine, Kyungpook National University, Daegu City 702-701; [2]Jinju Bio Food, Jinju City 660-931, Republic of Korea

Aging is an extremely complex, multifactorial process and represents the gradual deterioration in function that occurs after maturity and leads to disability or death. The examination of aging in vital organs including the liver is important for understanding the aging processes. However, the mechanism of age-dependent change in vital organ is still unclear. Therefore, age-dependent changes of the liver were examined using Senescene marker protein-30 knockout ($SMP30^{-/-}$) mice as an aging model. SMP30 has been reported as decreasing with age and functioning as gluconolactonases in L-ascorbic acid synthesis. $SMP30^{-/-}$ mice were more sensitive to apoptotic reagents and shorter in life span than normal mice. In microscopic findings of the liver from aged $SMP30^{-/-}$ mice without an ascorbic acid (AA) feeding for 16 weeks, there was an increased amount of both lipid droplets and glycogen in hepatocytes, than in those fed AA and hepatic stellate cells (HSCs) were hypertrophic with lipid droplets which indicated a hypervitaminosis A condition. Moreover, the level of peroxisome proliferators-activated receptor gamma (PPARγ) increased in the liver. Surprisingly, HSC hypertrophy of aged mice was decreased after treatment with ENA Actimineral A (ENA), which is alkaline bioactive water and has antioxidant function, as compared to non-treatment group. In conclusion, we propose that AA deficiency in aged $SMP30^{-/-}$ mice accelerate the hypertrophy of HSCs with vitamin A accumulation as well as the accumulation of lipid and glycogen in hepatocytes, and increase the expression of PPARγ.

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EFFECT OF ANGIOTENSIN II TYPE 1 RECEPTOR BLOCKADE ON SKELETAL MUSCLE INJURY BY TGF-BETA INDUCED FROM CCL_4 INJURED LIVER

Ok-Kyung Hwang[1], Jin-Kyu Park[1], Il-Hwa Hong[1], Mi-Ran Ki[1], Dong-Wei Yuan[1], Jung-Youn Han[1], Kyung-Sook Hong[1], Ae-Ri Ji[1], Ronald D Cohn[2], Soon-Hak Kwon[3] and Kyu-Shik Jeong[1]

[1]Department of Veterinary Pathology, College of Veterinary Medicine, Kyungpook National University, Daegu City 702-701, Republic of Korea; [2]McKusick-Nathans Institute of Genetic Medicine, Department of Pediatrics, Johns Hopkins University School of Medicine, 733 N Broadway, Baltimore, Maryland 21205, USA; [3]School of Medicine Kyungpook National University, Daegu City, Republic of Korea

Several therapeutic agents have been shown to inhibit fibrosis and improve regeneration after injury in skeletal muscle by antagonizing transforming growth factor-beta1 (TGF-β_1). Angiotensin receptor blockers have been shown to have a similar antagonizing effect on TGF-β_1 in a variety of tissues. In this study we evaluated the effect of Losartan, angiotensin II type 1 receptor blockade, on skeletal muscle injury via elevated TGF-β_1 produced by CCl_4 induced liver injury. Male C57BL/6 mice were randomly divided into three groups: control, CCl_4-treatment group (CT) and CCl_4-treatment group with adding losartan (CTL). 10% CCl_4 in olive oil were injected intraperitoneally three times a week with or without losartan (10 mg/kg) in drinking water. At 16 weeks, all mice were sacrificed and analyzed through the biochemistry, histopathology, immunohistochemistry and immunoblot tests. Expression level of creatine kinase (CK) and TGF-β_1 in serum were the highest in CT and their level significantly decreased in the losartan treated group. On immunohistochemistry and immunoblot Dystrophin, sarcolemma membrane structural protein, was significantly decreased in CT (49.3±2.8) group compared with CTL (87.3±8.5) group. p-Smad2/3 which is the downstreams of TGF-β_1 was significantly increased in CT (1216.3±105.5) group compared with CTL (547.3±52.5) group. Pax7, transcription factor of quiescent satellite cell was increased in CTL (0.63±0.03) group compared with CT (0.47±0.02) group. Myogenic regulatory factors including MyoD and Myogenin also showed the same results with pax7. In conclusion we suggest the skeletal muscle myogenesis was impaired by producing TGF-β_1 from CCl_4-induced liver injury and, angiotensin II type 1 receptor blockade (Losartan) has protective effect on TGF-β_1-induced skeletal muscle injury *via* the stimulation of muscle regeneration, indicating potential therapeutic effects in myopathies.

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ENA-ACTIMINERAL A EXTEND THE LIFE-SPAN OF SMP30 KNOCKOUT MICE *VIA* ANTI-OXIDANT MECHANISM

Jung-Youn Han[1], Jin-Kyu Park[1], Mi-Ran Ki[1], Dong-Wei Yuan[1], Il-Hwa Hong[1], Moon-Jung Goo[1], Ok-Kyung Hwang[1], Kyung-Sook Hong[1], Ae-Ri Ji[1],Yoo-Kyung Kim[2], Sung-Yong Hwa[3] and Kyu-Shik Jeong[1]

[1]Department of Pathology, College of Veterinary Medicine, [2]Department of Home Economics Education, Kyungpook National University, Daegu City 702-701; [3]Jinju Bio Food, Jinju City 660-931, Republic of Korea

ENA-Actimineral A (ENA), alkaline mineral water which is composed of refined edible cuttlefish (*Sepia esculenta*) and two different species of seaweed, such as *phymatolithon calcareum* and *Lithothamnion corallioides*, was previously reported to restore bone loss and bone quality in ovariectomized rats. To investigate the anti-oxidant effect of ENA, an experiment was performed with Senescence marker protein-30 knockout (SMP30 KO) mice as an aging model. SMP30 was known to maintain calcium homeostasis and up-regulate synthesis of vitamin C as an antioxidant in previous studies, therefore, SMP30 KO mice can be regarded as a good aging model. Present studies were composed of 18 week-old mice groups (n=24), 26 week-old mice groups (n=12) and 46 week-old mice groups (n=20). Each differently aged mice group was divided into three groups again: control group, 5%ENA and 10% ENA treated group. All groups of 18 week-old mice were treated with vitamin C 1.5g/L drinking water. The experiments were performed for 18 weeks. In the examination of life span, all vitamin C treated groups revealed 100% of survival rate. However, in non-vitamin C treated groups, only 10% ENA treated groups showed 100% of survival rate, as apposed to control group and 5% ENA treated groups, indicating 0% of survival rate. In vitamin C treated groups, the concentration of vitamin C in serum was increased by ENA dose-dependently. In TUNEL assay, a number of positive hepatocytes decreased significantly ENA dose-dependently. In immunoblot analysis of anti-oxidant protein expression, the expression level of Cu, Zn-SOD was increased by ENA dose-dependently. These data strongly suggested that intake of ENA plays a critical role in the anti-aging mechanism and it can act as a powerful anti-oxidant agent.

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NEUTRALIZING INTERACTIONS BETWEEN CYTOTOXIC DRUGS

H. Ehrhardt, L. Pannert, K. Schneider and I. Jeremias

Helmholtz Zentrum München, Marchioninistrasse 25, 81377 Munich and Department of Oncology / Hematology, Dr. von Haunersches Kinderspital, Lindwurmstr 4, 80337 München, Germany

Within polychemotherapy protocols, cytotoxic drugs are applied in combinations in order to overcome drug resistance and to enhance treatment efficiency. For this purpose, anthracyclines are frequently given on the same day as vinca-alkaloids, *e.g.* during induction therapy of acute lymphatic leukemia treatment.

Here we found that anthracyclines inhibited the antitumor activity of vinca-alkaloids both on tumor cell lines as well as on primary tumor cells of children with acute lymphatic leukemia. The inhibitory function of anthracyclines strongly depended on the activation of defined intracellular signaling steps and inhibited mitochondrial depolarization and activation of caspases by vinca-alkaloids. Of great clinical importance, the anthracycline-induced inhibition of vinca-alkaloid-mediated tumor cell death was absent if both drugs were given at certain intervals.

Our data show for the first time that cytotoxic drugs exert adverse, antiapoptotic effects on tumor cells which might be avoided in future polychemotherapy protocols using favorable application schedules.

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PHASE III RANDOMIZED STUDY COMPARING ORAL PILOCARPINE *VS.* SUBMANDIBULAR SALIVARY GLAND TRANSFER PROTOCOL FOR THE MANAGEMENT OF RADIATION INDUCED XEROSTOMIA

Naresh Jha, Hadi Seikaly, Jeffrey Harris, David Williams, Khalil Sultanum, Michael Hier, Sunita Ghosh, Martin Black, James Butler, Donna Sutherland, Paul Kerr and Pam Barnaby

Cross Cancer Institute, Edmonton, Alberta, Canada

Objective and Methods: We report results (median follow up 21 months) of a prospective phase III multicenter study comparing Pilocarpine with submandibular salivary gland transfer procedure for management of radiation induced xerostomia. Eligibility criteria included squamous cell carcinoma of Larynx, Hypopharynx, Oropharynx and unknown primaries with neck nodes. Patients with carcinoma of the Nasopharynx, Oral cavity, N3 disease, bilateral neck nodes, level 1 nodes or pre-epiglottic involvement were ineligible. Treatment strategies included either surgery as prime modality of treatment followed by chemoradiation treatment or Chemoradiation treatment as prime modality with or without planned neck dissection. Patients in Pilocarpine arm received Pilocarpine 5 mg. three times a day for 3 months. Salivary functions were evaluated by measuring salivary flows (baseline and stimulated) and quality of life using Univ. of Washington Quality of Life (QOL) Questionnaire, preoperatively, 1,3, 6, 12 and 24 months after treatment. Primary end point was the salivary functions (amount and consistency of saliva) at 6 months follow up. *Results:* This study was closed as per stopping rules, at interim analysis as the intent to treat analysis of all patients had shown significantly superior results in the gland transfer arm. The median follow up of these patients now is 21 months and results at 16 months follow up are presented. Significantly superior results are noted in the gland transfer arm as compared to Pilocarpine arm: for the median baseline and stimulated salivary flow, p=0.021 and 0.004, respectively, and for patients reporting none or minimal xerostomia [score 10 or 20 as per VII A (amount of saliva) of Univ. of Washington QOL, p=0.005 and for consistency of

saliva, p=0.008. *Conclusion:* Salivary gland transfer remains superior to Pilocarpine in the management of radiation induced xerostomia.

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GENETIC ASPECT OF GLIOBLASTOMA MULTIFORME: INVOLVEMENT OF mTOR COMPLEXES

Michael A Karsy, Nicholas Gulati, Ladislau Albert, Raj Murali and Meena Jhanwar-Uniyal

Department of Neurosurgery, New York Medical College, Valhalla, NY, 10595, USA

Human malignant glioblastoma multiforme (GBM) develops as the result of multiple stepwise genetic alterations, arising either *de novo* (primary GBM) or progressing from low-grade astrocytoma (secondary GBM). The genetic nature of GBM echoes the clinical dichotomy that exists between primary and secondary GBM. Mutation and/or deletion of the tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is one of the most common genetic alterations in GBM, with an estimated frequency of 70-90%. This suggests that the PTEN/PI3K/AKT signaling pathway plays a critical role in oncogenesis. Furthermore, PTEN loss increases the pool of self-renewing neural stem cells and induces loss of homeostatic control of proliferation, which is reminiscent of the cell-cycle dysregulation that occurs during gliomagenesis. Activation of the oncogene AKT due to PTEN loss stimulates the downstream kinase mammalian target of Rapamycin (mTOR), which has been shown to play a crucial role in cell survival, growth, and motility through two distinct multi-protein complexes: mTORC1 (a mammalian counterpart of TORC1) and mTORC2 (a mammalian counterpart of TORC2). Rapamycin (RAPA), an mTOR inhibitor, and its analogs have been considered potential candidates for the treatment of GBM. However, GBM still remains incurable. We hypothesize that the contributions of both mTORC1 and mTORC2 result in uncontrolled GBM growth and dissemination and therefore provide a molecular understanding of GBM. The interplay of mTORC1 and mTORC2 was studied in cell lines LN18, U87, and U373. Immunohistochemical analysis demonstrated activated AKT ($pAKT^{Ser473}$) expression in 85% of human tumor samples. GBM cells were treated with either RAPA or PI3K inhibitor LY294002 (LY) in the presence or absence of platelet-derived growth factor (PDGF) or extracellular matrix fibronectin (FN). Levels of phosphorylated AKT, p70S6K, and S6K, downstream proteins of the PTEN/PI3K pathway, as well as STAT3 and ERK1/2, proteins parallel to the PTEN/PI3K pathway, were assessed by immunoblotting. RAPA treatment resulted in sustained suppression of $pS6K^{Ser235/236}$ (mTORC1 substrate) through a 24h period. Treatment with RAPA noticeably reduced activation of AKT^{Ser473} (mTORC2 substrate) by 61% and 72% at 1 and 3 h, respectively, followed by enhanced activation up to 24 h. This finding suggests a shift between mTORC1 and mTORC2 following RAPA treatment. Pre-treatment with RAPA or LY diminished PDGF or FN stimulation of $p70S6K^{Thr389}$. A combined treatment with RAPA and LY completely blocked the activation of S6K. Activation of STAT3 by PDGF or FN was totally abolished by combined pre-treatment with RAPA and LY. Silencing of Rictor, a mTOR partner, inhibited activation of pAKT, a downstream component of mTORC2. While both motility and proliferation were found to be decreased at early time points (1-3 h) following RAPA treatment, later time points (6-24 h) showed enhanced motility and proliferation. Both cellular motility, as assessed by radial monolayer migration, and cellular proliferation, as analyzed by MTT assay, exhibited that the changes in cellular dynamics reflected an enhanced mTORC2 pathway. Treatment with RAPA caused an initial decrease in mTORC1 activity but then shifted to enhanced mTORC2 activity inducing AKT signaling. Thus, deciphering these molecular pathways would provide better understanding of gliomagenesis.

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MOLECULAR IMAGING OF *BCL-2*-POSITIVE AND -NEGATIVE LYMPHOMA XENOGRAFTS USING PEPTIDE-NUCLEIC ACID-PEPTIDE CONJUGATES

F. Jia[1], B.S. Balaji[1], S.D. Figueroa[5], F.Gallazzi[2], S.Z. Lever[3], M. Hannink[4], T.J. Hoffman[6] and M.R. Lewis[1,5]

[1]Department of Veterinary Medicine and Surgery, University of Missouri-Columbia, Columbia, MO, USA;
[2]Molecular Biology Program, University of Missouri-Columbia, Columbia, MO, USA;
[3]Department of Chemistry, University of Missouri-Columbia, Columbia, MO, USA;
[4]Department of Biochemistry, University of Missouri-Columbia, Columbia, MO, USA;
[5]Radiopharmaceutical Sciences Institute, Research Service, Harry S Truman Memorial Veterans' Hospital, Columbia, MO, 65201, USA

Objective: The *bcl-2* gene is overexpressed in non-Hodgkin's lymphoma (NHL). Two peptide nucleic acid (PNA)-peptide conjugates targeting bcl-2 mRNA were investigated for direct comparison of *bcl-2*-positive and -negative cell lines and xenografts. *Methods:* The human small lymphocytic lymphoma (SLL) cell lines Mec-1 and Ramos express comparable levels of somatostatin receptor subtype 2 for peptide nucleic acid-peptide delivery, but the Mec-1:Ramos

bcl-2 mRNA expression ratio was determined by RT-PCR to be 3821:1. The cell lines were used in cell efflux studies, and the corresponding xenografts were used for *in vivo* distribution and imaging studies in SCID mice. DOTA-anti-*bcl-2*-PNA-Tyr^3-octreotate was labeled with ^{111}In (**1**). DOTA-anti-*bcl-2*-PNA-Ts^{14}-Tyr^3-octreotate was labeled with ^{64}Cu (**2**) for *in vivo* studies because micro-PET with this agent gave superior imaging quality. *Results:* The cell associated radioactivity of conjugate **1** was 15% in Ramos cells, as compared with 60% in Mec-1 cells at 4 h of incubation. Biodistributions showed a 0.2% ID/g tumor uptake of conjugate **1** in Ramos mice and 1.3% ID/g in Mec-1 mice at 48 h post-injection. The conjugate **2** in Ramos mice showed a 0.7% ID/g tumor uptake and 1.1% ID/g in Mec-1 mice. Both conjugates could detect Mec-1 tumors by micro-SPECT and micro-PET, but not Ramos tumors. *Conclusion:* The significantly ($p<0.05$) higher retention of conjugate **1** in Mec-1 cells suggested specific bcl-2 mRNA targeting. Biodistribution and imaging studies also concluded that both conjugates **1** and **2** are specific for bcl-2 mRNA-positive tumor xenografts.

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YKL-40: A POTENTIAL NEW BIOMARKER IN CANCER PATIENTS

Julia S. Johansen

Herlev Hospital, University of Copenhagen, Denmark

YKL-40 (Chitinase-3-like-1, CHI3L1) is a 40 kDa heparin-, chitin- and collagen-binding glycoprotein without chitinase activity and a member of mammalian chitinase-like proteins. Its gene is known (1q32.1, 7948 base pairs, 10 exons), but *YKL-40* gene mutations and polymorphisms in cancer patients are not described. High YKL-40 mRNA and protein expressions are found in embryonic stem cells, embryonic and fetal cells, in macrophages, leucocytes and mast cells in areas with inflammation, in tumour-associated macrophages, and by several types of cancer cells including adenocarcinoma, squamous cell carcinoma, melanoma and glioblastoma. In some, but not all, types of cancer, YKL-40 protein expression in cancer cells is a biomarker of genetic and histological subtype, therapeutic response, and prognosis.

Few studies have evaluated the function of YKL-40 in cancer. It may have a role in proliferation, differentiation, metastatic potential, angiogenesis and response to hypoxia, and protection against apoptosis. YKL-40 may also play a role in the inflammatory response and extracellular tissue remodelling with development of fibrosis in the area surrounding the cancer cells. Receptors mediating its effects are unknown. YKL-40 is regulated by TNFα and IL-6, requires sustained activation of NF-kappaB, initiates MAP kinase and PI-3K signalling cascades, leading to AKT-mediated signalling cascades. Up-regulated YKL-40 expression is found in glioblastoma cells following stress stimuli, *e.g.* hypoxia, ionizing radiation and chemotherapy.

Plasma YKL-40 is elevated (*i.e.* above the age-adjusted 95th percentile of healthy individuals) and is related to tumour stage in some patients with different types of primary or metastatic solid cancer (breast, cervical, endometrial, glioblastoma, head and neck, kidney, small and non-small cell lung, melanoma, ovarian, pancreatic, prostate), and in patients with acute myeloid leukemia, multiple myeloma, and Hodgkin lymphoma. Highest plasma YKL-40 is found in patients with metastatic cancer, the shortest recurrence- or progression-free interval, and the shortest overall survival. YKL-40 is neither organ- nor tumour-specific, but plasma YKL-40 may be useful as a prognosticator of survival, and a predictor of treatment response. High plasma YKL-40 is an independent prognostic biomarker of short survival in patients with local or metastatic cancer, at time of first cancer diagnosis and at relapse, and is independent of other prognosticators (ER, HER2, CEA, CA125, LDH, CRP). High plasma YKL-40 predicts: 1) low response to anthracycline therapy in patients with first recurrence of breast cancer; and 2) second-line chemoresistance in ovarian cancer patients. Some studies suggest that plasma YKL-40 may be useful for monitoring cancer recurrence or progression after treatment. In the general population without known cancer, high plasma YKL-40 predicts a 3.4-fold increased risk of gastrointestinal cancer. In patients referred to endoscopy due to symptoms or other risk factors for colorectal cancer, plasma YKL-40 independently predicts colorectal cancer. This suggests a value for plasma YKL-40 in screening.

Since YKL-40 is not specific for cancer, it is important to consider co-morbidity and repeated measurements when evaluating plasma YKL-40 as a biomarker for cancer, and a normal plasma YKL-40 cannot rule out cancer. Plasma YKL-40 may have a place as a biomarker in patients with cancer and provides new information compared to rutinely used biomarkers, but more studies are needed. However, YKL-40 is probably more than a biomarker. A major issue to explore is the question if YKL-40 could become a target for the development of new cancer therapeutics.

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CONNECTIONS BETWEEN G-QUADRUPLEXES, THE WERNER AND BLOOM SYNDROMES, AND CELLULAR SENESCENCE

Li-San Wang[1,5,6], Steven Hershman[4], Jasmine S. Smith[1,2], Jay E. Johnson[1,5], Marina L. Kozak[1,2], Kajia Cao[1], Paul Ryvkin[3], Qijun Chen[1,5] and F. Brad Johnson[1,2,5]

[1]Department of Pathology and Laboratory Medicine, [2]Cell and Molecular Biology Graduate Program, [3]Genomics and Computational Biology Graduate Program, [4]Vagelos Scholar Program, [5]Institute on Aging,

[6]Penn Center For Bioinformatics, University of Pennsylvania, Philadelphia, PA 19104, USA

G-quadruplex DNA (G4-DNA) is a family of structures composed of stacked G-quartets, which themselves comprise four Hoogsteen-bonded guanines in a planar array. They have been shown to form *in vivo* at telomeres in at least one organism, *S. lamnae*. Several RecQ DNA helicases, including the human WRN and BLM proteins and *S. cerevisiae* Sgs1, are particularly active in unwinding G4-DNA *in vitro*. WRN and BLM are deficient in the Werner and Bloom syndromes, respectively, which are characterized by elevated rates of cancer and premature features of aging. The demonstrated roles for RecQ helicases in telomere maintenance has led to the hypothesis that these roles might be explained by the resolution of telomeric G4-DNA by the helicases. New experimental findings will be presented that support this idea directly.

Outside of telomeres, sequences with intramolecular G-quadruplex forming potential (QFP) are concentrated in the promoter regions of organisms ranging from bacteria to humans, raising the possibility that they play roles in transcriptional regulation. Recently we reported highly significant correlations in *S. cerevisiae* between genes having QFP and those with altered expression in cells lacking Sgs1 or treated with a selective G4-DNA small molecule ligand N-methyl mesoporphyrin IX (NMM), thus supporting a direct role for G4-DNA in transcriptional regulation. We have now extended these findings to human cells, including analyses of altered gene expression in cells from individuals with Werner or Bloom syndrome, or in cells treated with NMM or another G4-DNA ligand, BRACO19. Similar to our findings in yeast, there are significant correlations between loci possessing QFP and those showing altered regulation under these conditions, each of which is predicted to alter G4-DNA levels or functions.

We wondered if there might be connections between G4-DNA at telomeres and that involved in transcriptional regulation. Cellular senescence can be induced by telomere shortening and dysfunction in both *S. cerevisiae* mutants lacking telomerase and human cultured cells. We have found, in both yeast and human cells, that there are highly significant correlations between genes that are upregulated at senescence caused by telomere shortening and genes with QFP. These associations are apparently not explained by the binding of known transcription factors. We therefore propose that 1) there is competition between G4-DNA at telomeres and promoters for transcriptional regulators that bind G4-DNA and 2) altered G4-DNA levels at the telomeres of senescent cells perturbs this competition, thus providing a novel mechanism for the regulation of gene expression in senescent cells.

Given the connections between cell senescence, telomere function, and RecQ helicases in cancer and age-related diseases, it may be possible to use manipulation of G4-DNA as a tool to impact these disorders.

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IMMUNOMODULATORY CAPACITY OF MYELOMA-DERIVED CHEMOKINE CCL27

Karin Jöhrer[1], Angelika Olivier[1], Michael Unger[1], Eva Maizner[1], Daniel Neureiter[2] and Richard Greil[1,3]

[1]Tyrolean Cancer Research Institute, Innrain 66, A 6020 Innsbruck;
[2]Institute of Pathology at the Private Medical University Hospital Salzburg, Müllner Hauptstrasse 48, A 5020 Salzburg;
[3]3rd Medical Department with Hematology, Medical Oncology, Hemostaseology, Rheumatology and Infectious Disease at the Private Medical University Hospital Salzburg, Müllner Hauptstrasse 48, A 5020 Salzburg, Austria

Multiple myeloma is still incurable using conventional chemotherapy and considerable efforts are undertaken to establish new immunotherapeutic strategies to target this B-cell neoplasm. Chemokines have currently been shown to be major players in shaping the tumor microenvironment and to contribute to immune escape of the malignant cells.

In order to reveal important actors of the chemokine network in multiple myeloma we analyzed the chemokine profile of myeloma cell lines. We found CCL27, which has so far only been correlated with skin diseases such as atopic dermatitis, consistently upregulated in all cell lines investigated. In bone marrow supernatants of tumor patients CCL27 expression correlated with the severity of disease. Additionally, immuno-histochemical analysis of patient bone marrow showed that CCL27 is expressed by the malignant cells and also highly expressed (or presented) by human bone marrow endothelial cells, the main barrier of the favourite myeloma microenvironment, the bone marrow. We further investigated the impact of CCL27 on immune cells such as T-cells and dendritic cells. Dendritic cells differentiated and matured in the presence of CCL27 exhibited a reduced capacity to activate T-cells in allogeneic mixed leukocyte reactions. On T-cell side, in this setting, proliferation as well as cytokine production was impaired. Treated dendritic cells showed normal expression of costimulatory molecules but impaired spontaneous migration as well as cytokine production which might explain the impaired T-cell function. In coculture experiments with myeloma cell lines, however, these dendritic cells induced enhanced growth of the malignant plasma cells. Since dendritic cells in our hands did not express relevant amounts of the cognate receptor for CCL27, *i.e.* CCR10, we postulate that the ligand is bound by glycosaminoglycans or by a so far unknown receptor. Binding studies are currently underway.

In summary, we found that the myeloma-derived chemokine CCL27 can modulate dendritic cells resulting in impaired activation of T-cells and enhanced tumor cell growth thus

contributing to worse prognosis. Targeting CCL27 therefore could constitute an essential additional component in myeloma therapy.

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BIRTH ORDER EFFECT IN THE INHERITANCE OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

Viggo Jønsson

Department of Hematology, Aker University Hospital, University of Oslo, NO 0514 Oslo, Norway

Birth order effect denotes a rank order of affected offspring in a sibship. In CLL, a birth order effect in affected parent-offspring pairs can reflect epigenetic factors in disease susceptibility. Thirty-two families segregating CLL and other lymphoproliferative disorders in a pleiotrophic pattern were identified. By means of Haldane Smiths's test and Cox regression it is shown that a paternal-offspring, but not a maternal-offspring birth rank order exists: In matrilineal transmission, affected offspring occur randomly in the sibship while in patrilineal transmission, mainly sons late in the sibship are affected. Cox regression analysis provided relative risks (RR) for paternal and maternal transmission of 3.60 (95% CI: 1.54-8.42; p=0.0005) and 1.64 (95% CI: 0.90-3.01; p=0.096), respectively. The significance of paternal and maternal transmission in CLL-CLL pairs employing Haldane and Smith's test were 0.006 and 0.63 respectively. There was no evidence of a relationship between parental age and birth order. Most likely, the birth order effect is a non-Mendelian segregation of the CLL monoallelic poly-susceptibility genes based on parental imprinting and pregnancy related microchimerism, where different combinations of the monoallelic polygenes code for the subtypes of lymphoproliferative disease, such as CLL, non-Hodgkin's lymphoma and Hodgkin's disease, the acute- and chronic lymphocytic leukemias and myeloma. CLL The impact on time to treat and intention to treat is discussed.

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HUMAN CANCER AND GENE MUTATIONS IN O-GLYCOSYLATION PATHWAY

Tongzhong Ju[1], Grainger S. Lanneau[2], Tripti Gautam[3], Yingchun Wang[1], Baoyun Xia[1], Sean R. Stowell[1], Margaret T. Willard[1], Wenyi Wang[1], Jonathon Y. Xia[7], Rosemary E. Zuna[4], Zoltan Laszik[4], Doris M. Benbrook[2,3,6], Marie H. Hanigan[5,6] and Richard D. Cummings[1]

[1]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA30322;
[2]Department of OB/GYN, [3]Department of Biochemistry and Molecular Biology, [4]Department of Pathology, [5]Department of Cell Biology, [6]Cancer Institute, The University of Oklahoma Health Sciences Center, [7]Oklahoma School of Sciences and Mathematics, Oklahoma City, OK73104, USA

Tn and Sialyl Tn (STn) antigens are tumor-associated carbohydrate antigens (TACAs) expressed by at least 60~70% of human carcinomas, including breast, colon, cervical, ovarian, and pancreatic cancer. Normally, Tn antigen is modified by Core 1 β3GalT (T-synthase), a key enzyme in mucin type O-glycosylation, to form the Core 1 structure, also called T-antigen. The T-antigen can be further elongated to form complex O-glycans on many cell membrane glycoproteins and secreted glycoproteins. Recently, we have identified that Cosmc, the Core 1 β3GalT-specific molecular chaperone, is required for active T-synthase formation. *Cosmc* is located on Xq24 and encodes an ER-localized type-II membrane protein that prevents aggregation/proteasomal degradation of T-synthase. Although it has been reported that Tn and STn antigen expression in human tumors are associated with poor prognoses, the genetic basis for their expression in human tumors, as well as the benefit for tumor cells expressing Tn/STn antigens are not known. We have now found that all tumor cell lines expressing the Tn and STn antigens have acquired mutations in *Cosmc*. The mutations are nucleotide deletions or insertions in the coding region, or deletions in the promoter sequence. In all cases, the mutations in the *Cosmc* gene result in loss-of-function for Cosmc as a chaperone and lead to aggregation and enhanced degradation of the T-synthase. Expression of wild-type Cosmc restores the T-synthase activity and cells lose expression of both the Tn and STn antigens. In addition, human cervical cancer specimens that showed expression of the Tn/STn antigens were also found to have mutations in *Cosmc* and loss-of-heterozygosity for the X-linked *Cosmc* locus. Importantly, tumor cells with mutated *Cosmc* were less sensitive to TRAIL-induced apoptosis than wild-type Cosmc transfected cells. This is the first example of somatic mutations in multiple types of cancers that cause global alterations in cell surface carbohydrate antigen expression. Additionally, this is the first evidence of an advantage for tumor cells expressing Tn antigen on the surface. The understanding of the genetic basis of the unusual expression of Tn and STn antigens in human tumors will shed new light on the development of new diagnostic, prognostic, and potentially therapeutic methods.

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COMPLEX MECHANISMS OF REGULATION OF PLATELET-DERIVED GROWTH FACTOR-INDUCED ERK ACTIVATION

Aleksandra Jurek, Carl-Henrik Heldin and Johan Lennartsson

Ludwig Institute for Cancer Research, Uppsala University, Box 595, SE-751 24 Uppsala, Sweden

Extracellular signal regulated kinases (Erks) represent a signalling hub in many physiological responses and have critical functions in cell proliferation, differentiation, survival and apoptosis. The physiological outcome of Erk signalling depends on both the magnitude and duration of kinase activation which are tightly controlled by many factors like the association of Erk with scaffolding proteins, cross-talk with other signalling pathways and negative regulators such as protein phosphatases. In this study we investigated the mechanisms regulating the Erk1/2 activation downstream of platelet-derived growth factor receptor α and β. Particularly, we were interested in evaluating an involvement of MAP kinase phosphatases, SHP2, PLCγ, Src and PI3K in the regulation of the Erk phosphorylation pattern. We found that MAP kinase phosphatase 3 is an important regulator of the PDGF-induced Erk activation acting in both a rapid positive feed-forward and a later negative feed-back loop. In addition, our results suggest an implication of SHP2 and Src in the modulation of the strength of Erk phosphorylation. As the Erk signalling pathway is up-regulated in many cancer cells, understanding of the complex mechanism of its regulation is of key importance for therapeutic approaches.

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ANTITUMOR ACTIVITY OF (–)-EPIGALLOCATECHIN-3-O-GALLATE FATTY ACID DERIVATIVES

Kunihiro Kaihatsu[1], Kazuaki Matsumura[2], Shuichi Mori[1], Han Hee Cho[2], Suong Hyu Hyon[2] and Nobuo Kato[1]

[1]The Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka, 567-0047; [2]Institute for Frontier Medical Sciences, Kyoto University, 53 Kawahara-Cho Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan

(–)-Epigallocatechin-3-O-gallate(EGCG), a major green tea catechin component, has been reported to possess antitumor activity. We developed a method to increase chemical stability and cell membrane affinity of EGCG using lipase-catalyzed trans-esterification. EGCG-fatty acid monoesters showed marked antitumor activities *in vitro* and *in vivo* by apoptosis induction through inhibition of epidermal growth factor receptor phosphorylation.

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MGMT, P53 AND DNA DOUBLE-STRAND BREAK REPAIR IN ALKYLATING DRUG-INDUCED APOPTOSIS: IMPLICATIONS FOR GLIOMA THERAPY

Bernd Kaina, Luis Z. Batista, Theodora Nicolova and Wynand P. Roos

Institut für Toxikologie, Mainz, Germany

An important group of anticancer drugs is represented by methylating and chloroethylating agents that are being used in first-line therapy of gliomas and malignant melanomas. These agents induce a dozen DNA lesions, some of them have been identified to be carcinogenic, genotoxic and cytotoxic. In cells lacking the repair enzyme MGMT the DNA adducts O^6-methylguanine (O^6MeG) and O^6-chloroethylguanine (O^6ChlG) are the major triggers of genotoxicity and apoptosis. In the case of O^6MeG lesions, this requires MSH2/MSH6-dependent mismatch repair. O^6MeG triggered cell death is regulated in a cell type specific manner, utilizing both the mitochondrial and the death receptor pathway. O^6MeG triggered apoptosis is preceded by DNA double-strand break (DSB) formation, H2AX phosphorylation and ATM/ATR activation. In turn, in p53-wt-expressing glioma cells, the Fas/CD95/Apo-1 receptor pathway becomes activated. In p53-mutated glioma cells, a hallmark of O^6MeG triggered apoptosis is decline of Bcl-2, which is followed by caspase-9, -7 and -3 activation. The efficiency of O^6MeG in triggering the p53-dependent (Fas) pathway is much higher than the p53-independent endogenous mitochondrial pathway, which explains why p53-wt glioma cells are more sensitive to TMZ than p53-mutated cells. Interestingly, p53-wt glioma cells are more resistant than p53-mutant glioma cells to chloroethylating agents, such as BCNU and ACNU, indicating p53 protects against O^6ChlG-induced apoptosis and necrosis, very likely by up-regulation of repair genes (notably *ddb2*). O^6MeG and O^6ClG are also able to induce apoptosis in malignant melanoma cells, which is accompanied by the formation of DSBs. Cells defective in XRCC2 or BRCA-2 are hypersensitive to O^6MeG-triggered cell death and chromosomal aberrations while DNA-PKCS mutated cells display only slightly enhanced sensitivity. The data supports a role for DSBs as most critical downstream apoptotic lesions and DSB repair by homologous recombination as a potential predictor of cellular resistance to monofunctional alkylating drugs.

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DOSE-EFFECT RELATIONSHIPS FOR CATARACT INDUCTION AND LATE RENAL DYSFUNCTION FOLLOWING TBI AND RECURRENCE OF KELOID AND PTERYGIUM FOLLOWING SURGERY AND RADIOTHERAPY

Henk B. Kal, M. Loes van Kempen-Harteveld, Ronald E. Veen, and Ina M. Jürgenliemk-Schulz

Department of Radiation Oncology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands

Purpose: To demonstrate radiation dose-response relationships for cataract induction and late renal dysfunction following

total body irradiation (TBI) and hematopoietic stem cell transplantation (HSCT) in patients with haematological malignancies, and for recurrence of keloid and pterygium after surgery followed by radiotherapy. *Materials and Methods:* A retrospective review using Pubmed was performed of articles reporting dose-response relationships for cataract induction and late renal dysfunction following TBI and recurrence of keloid and pterygium postoperatively following radiotherapy. The radiation regimens identified were normalized using the linear-quadratic model; biologically effective doses (BEDs) were calculated. *Results:* For cataract induction a dose-effect relationship after TBI and HSCT for acute leukaemia was found. A threshold BED of about 45 Gy was indicated. For late renal toxicity after TBI and HSCT, the threshold BED is about 16 Gy. For keloid recurrence after keloidectomy followed by radiotherapy either with teletherapy or brachytherapy, the recurrence rate following a BED >30 Gy was less than 10%. For pterygium recurrence following bare sclera surgery and ^{90}Sr beta irradiation, a BED of about 30-40 Gy seems to be sufficient to reduce the recurrence rate to less than 10%. *Conclusion:* Dose-response relationships were identified for cataract induction and late renal toxicity after TBI, as well as for keloid and pterygium recurrence after surgery and radiotherapy. To prevent cataract induction, eye shielding should be applied for single-dose and hypofractionated TBI. For almost all TBI schemes in use, kidney shielding should be applied. Most radiotherapy schemes used for keloid recurrence prevention after surgery are not sufficient. A minimal dose with a BED of 30 Gy should be applied. In contrary, the doses applied in most regimens to prevent pterygium recurrence are too high. A scheme with a BED of 30-40 Gy seems to be sufficient.

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MOLECULAR MECHANISMS OF CALCIUM-MEDIATED ANTIMITOGENIC ACTIONS IN COLON CANCER CELLS

Enikö Kallay

Department for Pathophysiology, Center for Physiology, Pathophysiology, and Immunology, Medical University of Vienna, Austria

Epidemiological, experimental, and animal studies indicate that theoretically, at least 50% of sporadic colorectal cancer could be prevented by modifications in diet. Primary prevention of cancer is, therefore, rational, timely and important, and should be a major priority and responsibility for international agencies, governments, industry, non-governmental organizations, medical and health authorities. In spite of the already significant amount of evidence there is still reluctance to accept that diet has a fundamental role in colon tumourigenesis, due to poor understanding of the mechanisms that regulate this process. Analysis of gene expression profiles provides important insights into how diets may alter metabolic profiles and regulatory pathways that influence probability of tumour formation in the normal intestinal mucosa. Evidence is accumulating for a prophylactic effect of calcium in colorectal cancer development. It has been show that a diet rich in calcium reduces crypt cell hyperproliferation both in human and animal studies. Calcium-mediated signal transduction may play a central role in hyperproliferation, transformation, invasion and metastasis. Effect of extracellular calcium could involve signal transduction through guanine nucleotide binding proteins, such as the calcium-sensing receptor (CaR).

Analysing the effect of extracellular calcium on colon cancer cell lines showed that calcium had a significant effect on genes implied in several different functional categories: Genes involved in cell cycle regulation and nucleic acid synthesis were down-regulated by calcium treatment, consistent with the parallel induction of cell cycle arrest. Coordinate down-regulation of genes involved in RNA processing, translation, and protein degradation were also evident. Conversely, genes involved in xenobiotic and drug detoxification, were up-regulated by calcium. The earliest changes due to calcium treatment involved tight junctions, focal adhesion, adherens junctions, with some of the parameters being up- others down-regulated. A series of known CaR signalling functions and pathways have been affected by calcium treatment, amongst them expression of genes coding for G alpha q, PLC, PLA_2.

Our data is further proof for the antiproliferative effect of calcium in colonocytes, suggesting that the CaR has a major role in colon tumourigenesis.

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HOW DO TUMOR CELLS REPROGRAM TUMOR-ASSOCIATED BRAIN MACROPHAGES INTO INVASION-PROMOTING CELLS AND EVADE IMMUNE RESPONSES?

Bozena Kaminska, Pawel Wisniewski, Maciej Lipko, Aleksandra Ellert-Miklaszewska and Michal Dabrowski

The Nencki Institute of Experimental Biology, Warsaw, Poland

Interactions between tumor-infiltrating leukocytes and tumor cells have been of great interest due to the possibility that immune cells either interfere with tumor progression or actively promote tumor growth. Microglia (brain macrophages) are multifunctional, innate immunity cells executing protective functions under physiological conditions. Intracellular inflammatory signaling pathways

include NFκB and MAP kinases, as critical signal transducers. On the other hand, the most invasive and dangerous brain tumors, malignant gliomas, recruit numerous microglia to the tumor site. Molecular mechanisms underlying accumulation and activation of tumor-associated microglia are unknown. We demonstrated that soluble factors released by glioma cells convert brain macrophages (microglia) into amoeboid/phagocytic cells which support glioma invasion, while attenuating inflammatory responses (Sliwa *et al*., BRAIN 2007; Wesolowska *et al*., Oncogene 2007). To identify those factors, we employed a proteomic approach and mass-spectrometry in search for fractions of glioma-conditioned medium (G-CM) able to induce morphological transformation of microglia. We identified osteopontin and lactadherin, two proteins sharing the RGD motif and known to interact with integrins. Interference with integrin binding using RGD-containing peptide completely abolished glioma-induced transformation of microglia and signal transduction. Analyzing signal pathways and global gene expression, we demonstrated that glioma-derived factors induce an alternative, non-immune activation of microglia. While lipopolysaccharide-induced activation involved NFκB and MAPKs activation, glioma-derived factors preferentially induce integrin-linked and TGFβ-driven signaling. Increased phosphorylation of FAK, pro-survival Akt kinase, JNK and p38 MAPK and stimulation of STAT3/5 were followed by release of anti-inflammatory cytokines and metalloproteinases stimulating motility, and invasiveness of glioma cells. Gene profiling and computational analysis revealed that the most discriminative feature was a lack of interferon-responsive genes in glioma-exposed microglia that may explain a lack of effective antitumor immune response. Our findings suggest that gliomas attract brain macrophages and employ specific signals, to transform them into tumor-supportive cells, We show for the first time molecular signals and mechanisms responsible for "re-education" of tumour-associated microglia into tumour supportive cells.

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DOWN-REGULATION OF *PLAKOGLOBIN* IN SOFT TISSUE SARCOMA IS ASSOCIATED WITH A HIGHER RISK OF PULMONARY METASTASIS

Yoshimitsu Kanazawa[1], Yoshimichi Ueda[2], Miyako Shimasaki[2], Shogo Katsuda[2], Norio Yamamoto[1], Katsuro Tomita[1] and Hiroyuki Tsuchiya[1]

[1]Department of Orthopaedic Surgery, Graduate School of Medical Science, Kanazawa University, 13-1, Takara-machi, Kanazawa, Ishikawa, 920-8641;

[2]Department of Pathology, Kanazawa Medical University, 1-1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa 920-0293, Japan

Soft tissue sarcomas (STS) behave with aggressiveness and metastatic potential, which can vary depending on their locations. There has been little information on the exact molecular mechanisms involved in their biological aggressiveness. To identify genes involved in the differences, the gene expression profiles were compared between STS-orthotopic and heterotopic implanted models, and their significance in human STS was verified. Human fibrosarcoma HT1080 cells were implanted either in the quadriceps femoris muscles or footpads of nude mice, and the gene expression profiles of the tumors were compared by cDNA arrays. The mRNA and protein levels of the identified genes were examined by both real-time RT-PCR and immunohistochemistry, not only in the tumors of the models, but also in clinical STS. The implanted HT1080 cells demonstrated different growth and metastatic potentials depending on their implant locations. cDNA array analyses showed reduced expression of the *plakoglobin* gene in the intramuscle-implanted group, which was statistically confirmed by real-time RT-PCR (p=0.04). Plakoglobin was immunolocalized diffusely in the cytoplasm of tumor cells implanted in the footpads, but not those in the muscle. Real-time RT-PCR assays of clinical STS showed that the mean *plakoglobin/G3PDH* ratio in primary sarcoma tissues with pulmonary metastases (0.92) was significantly lower than in those without metastasis (6.58) (p<0.0001), and that STS cases with high *plakoglobin* gene expression had an excellent prognosis. These results suggest that *plakoglobin* gene expression level might be useful as a new biomarker for metastasis and prognosis of human STS.

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MOLECULAR TARGETS IN BREAST CANCER THERAPY: A DIAGNOSTIC APPROACH

Nikiforos Kapranos

Department of Molecular Pathology, General, Maternity and Children's Hospital, Athens, Greece

Targeted therapy is a rapidly evolving field in breast cancer treatment. The most important therapeutic target is the ErbB family of receptor tyrosine kinases, especially ErbB2 which is inhibited by the widely used humanized monoclonal antibody trastuzumab. Although trastuzumab is successfully used in metastatic or adjuvant clinical settings, the accurate molecular testing of ErbB2 is still a major problem in the pathology laboratory. ErbB2 protein detection by immunohistochemistry is subjective and technically inaccurate, due to the unstable nature of proteins and the nonstandardised procedures in the

routine pathology laboratory. Immunohistochemical detection of ErbB2 remains, however, the basic technique for the selection of patients suitable for an expensive biological therapy and gene testing is reserved only for a small group of cases with inconclusive results. Fluorescence *in situ* hybridization (FISH) testing was initially used for the detection of *ErbB2* gene amplification, but this technique has a low discriminative ability in tissues, a temporary signal and requires special and expensive equipment. Chromogenic *in situ* hybridization (CISH) is a powerful, low cost alternative method for *ErbB2* gene analysis which offers a permanent signal and obvious morphological details under the usual bright field microscope. In combination with chromosome 17, ErbB2 CISH analysis provides important information concerning the presence of true gene amplification or protein overexpression due to chromosomal polysomy. The CISH technique, according to a recent international multicenter quality assurance study, was found to have excellent reliability and reproducibility. Finally, CISH may be supplemented by *topoisomerase IIa* gene analysis and provide further valuable information of prognostic and therapeutic significance.

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GENETIC PROFILING IN TUMOR XENOGRAFTS FROM PAEDIATRIC GLIOMAS BY EXPRESSION AND HIGH RESOLUTION COMPARATIVE GENOMIC HYBRIDISATION MICROARRAYS

Katherine Karakoula[1], Nicola Potter[1], Steven T. Keir[2], Darell D. Bigner[2], Kim P. Phipps[3], William Harkness[3], Richard Hayward[3], Dominic Thompson[3], Thomas S. Jacques[4,5], Brian Harding[5], David G.T. Thomas[6] and Tracy J. Warr[1]

[1]Department of Molecular Neuroscience, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, London, UK;
[2]Departments of Surgery and Pathology, Duke University Medical Center, Durham, USA;
[3]Department of Neurosurgery, Great Ormond Street Hospital for Children NHS Trust, London;
[4]Neural Development Unit, Institute of Child Health, University College London, London;
[5]Department of Histopathology, Great Ormond Street Hospital for Children NHS Trust, London;
[6]National Hospital for Neurology and Neurosurgery, London, UK

Gliomas are the leading cause of cancer-related mortality in children, with survival after diagnosis for the most aggressive subtype, glioblastoma multiforme (GBM), being less than 12 months in most cases. Improving survival rate in paediatric brain tumor patients continues to be a challenge, and for this, we still need to understand the molecular pathways involved in the genesis and progression of these types of cancer. DNA copy number changes represent molecular fingerprints of solid tumours and are as such relevant for better understanding of tumour development and progression. Gliomas are characterized by changes in the expression of genes, including amplification of oncogenes and loss of tumor suppressor genes, often as a result of genomic copy number alterations. In order to identify recurrent chromosomal regions of gain and loss, and thereby novel gene targets of potential importance for paediatric glioma development and/or progression, we have analyzed DNA copy number changes in ten tumor xenografts from paediatric gliomas, comprising 7 glioblastoma multiforme (GBM), 2 ependymomas (Grade II) and 1 primitive neuroectodermal tumor (PNET). The xenograft models were developed in athymic nude mouse (*nu/nu*) by subcutaneous implantation (Duke University, Medical Center). Gene expression profiles for tumour xenografts were generated using the Affymetrix U133A plus 2.0 arrays whereas genomic copy number changes were determined by high-density oligo microarray-based comparative genomic hybridisation (244A array-CGH). Results revealed frequently overexpressed (≥3-fold, $p<0.05$) genes in chromosomes with gains of 1, 2, 7, 8 and 20 and regions 12q and 13q. Furthermore, genes with high-level expression (≥10-fold) showed amplification. Similarly, deleted genes on chromosomes 3, 4, 6, 14 and 18 and regions 9p and 11q were associated with reduced expression (≥2-fold, $p<0.05$) in the tumour samples when compared to normal, brain controls. However, a number of genes with normal copy numbers showed reduced expression levels (≥2-fold, $p<0.05$). High resolution analysis allowed us to identify changes in expression of genes located within regions of copy number alterations in paediatric gliomas that could play an important role in tumour pathology or provide potential new therapeutic targets.

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GENE EXPRESSION AND STRUCTURAL MODIFICATIONS OF MATRIX MACROMOLECULES IN SOLID CANCERS – MOLECULAR TARGETS

N. Karamanos, O. Kousidou, A. Roussidis, Ch. Malavaki and P. Dedes

Laboratory of Biochemistry, Department of Chemistry, University of Patras, 261 10 Patars, Greece

Altered expression of effective matrix macromolecules by cancer cells is related to tumour growth, invasive properties and metastatic potential. The structural alteration and degradation of extracellular matrix (ECM) by neoplastic cells is a prerequisite for invasion and the formation of tumour metastases by the malignant cells. Differential synthesis of proteoglycans (PGs) in stroma and epithelial cells has been

related to breast tumourigenesis and proliferation, differentiation and the invasive properties of human epithelial breast cancer. Tumor cells can release metalloproteinases (MMPs) that degrade the matrix macromolecules and can therefore invade through tissue barriers, blood vessel and lymph channel walls. Modulation of MMP synthesis by cell–cell contact has been suggested to be a crucial event for enhancing the ability of breast cancer cells to invade the bone marrow fibroblasts. Conventional chemotherapy regimens for the treatment of breast cancer have limited efficacy and are associated with significant toxicity, highlighting the need for novel targeted therapies.

In order to examine whether gene expression of PGs and MMPs is related to growth and functional invasive potential, we performed *in vitro* studies on a panel of epithelial of breast (ER-positive and –negative) and colon cell lines in the presence of a series of potential inhibitors. The obtained results clearly showed that PI3/Akt and ERK1/2 signalling pathways are activated and that breast cancer is associated with significant changes in gene expression of secreted PGs as well as cell surface PGs, the expression of which is related to the balance between ERα/β.

Significant changes in gene expression of MMPs and their endogenous tissue inhibitors (TIMPs) were also found. The expression of certain types of MMPs can be related to the invasive properties of the epithelial breast and colon cancer cells. Studies to elucidate whether the modified gene expression of PGs, MMPs and TIMPs are associated with certain molecular targets and their respective signalling pathways, using specific tyrosine kinase inhibitors, such as STI571 (glivec), the phytoestogen genistein, the specific P450 aromatase inhibitor letrozole, as well as siRNA for the ER isoforms, showed that both tyrosine kinase pathways and status of ERs are key partners and of crucial importance for modified gene expression of matrix effectors and cancer cell growth.

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INVESTIGATION OF THYMIDYLATE SYNTHASE (TS) AND TOPOISOMERASE-I EXPRESSION IN PATIENTS WITH RESECTED COLORECTAL CANCER (RCRC) TREATED WITH ADJUVANT CHEMOTHERAPY. A RETROSPECTIVE STUDY OF THE HELLENIC COOPERATIVE ONCOLOGY GROUP (HECOG)

I. Kostopoulos[1], V. Karavasilis[1,2], M. Karina[1,2], M. Bobos[1], N. Xiros[2], C. Petraki[2], G. Pentheroudakis[2], G. Kafiri[2], P. Papakostas[2] and G. Fountzilas[1,2]

[1]Aristotle University of Thessaloniki, School of Medicine, Thessaloniki;
[2]Hellenic Cooperative Oncology Group (HeCoG), Athens, Greece

Aim: Thymidylate Synthase (TS) and Topoisomerase-I (Topo-I) are reasonable chemotherapeutic targets in CRC. We aimed to study the expression of TS and Topo-I in patients with rCRC who received adjuvant chemotherapy and correlate it with the clinical outcome. *Patients and Methods:* All patients, who were diagnosed with rCRC between 1989 and 2004 and treated with adjuvant chemotherapy within HeCOG's protocols, were identified from our electronic database. Archival FFPE tissue blocks obtained from surgical resection specimens of invasive colorectal adenocarcinoma were arrayed in multiple TMA blocks. IHC was performed on the TMA slides using monoclonal antibodies against the antigens TS and Topo-I. The immunocomplex was visualized using a polymer detection system and DAB as a chromogen. As external control of the immunoreaction a known positive and negative controls was used. In each slide was assigned a score for intensity and staining pattern using a 4-tier range system. The results were correlated with survival (OS) and disease free survival (DFS) in the view of irinotecan based chemotherapy. *Results:* A cohort of 498 cases with median age of 61 years, classified as Dukes stage B (49%) and C (51%), fulfilled the criteria of the study. In 59% of the cases the tumor was located in the rectosigmoid area. All patients received adjuvant 5-FU based chemotherapy and 35% were also treated with irinotecan, which is a target for Topo-I. TS and Topo-I expression was found in 43% and 48% of the cases respectively. Five- years OS was 74% and DFS was 68%. There was no correlation of TS and Topo-I expression with OS and DFS. The subgroup of patients who expressed Topo-I and treated with irinotecan had a better OS (HR=0.47, 95%CI 0.23-0.94, p=0.033). In irinotecan treated patients TS expression was correlated with shorter DFS (HR=1.50, 95% CI 1.03-2.19, p=0.036). *Conclusion:* Our data indicate that patients with rCRC with elevated levels of Topo-I protein may benefit from irinotecan containing adjuvant chemotherapy. However, randomized prospective trials are warranted to confirm these results.

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THE ANTITUMOR EFFECT OF LIPOSOME-ENCAPSULATED CISPLATIN ON RAT OSTEOSARCOMA AND ITS ENHANCEMENT BY CAFFEINE

Michiaki Karita, Hiroyuki Tsuchiya and Katsuro Tomita

Department of Orthopaedic Surgery, Graduate School of Medical Science, Kanazawa, Japan

Background: Liposomes have been proposed as useful drug carriers in targeted drug delivery system and are under investigation in several therapeutic fields. Caffeine has been identified as belonging to the group of xanthines that enhance the action of cisplatin. *Materials and Methods:* In this study,

liposomes containing polyethylene glycol encapsulated cisplatin (CDDP-L) were prepared. The action of CDDP-L on rat osteosarcoma and the enhancing action of caffeine on the antitumor effect of CDDP-L were evaluated. Using osteosarcoma-bearing rats, the retention of CDDP-L in the blood, its intratumor concentration, cytoreductive effect and the enhancing action of caffeine on its antitumor effect were examined. *Results:* The liposomes were able to remain in the systemic circulation for a long time and to be concentrated in the osteosarcoma, but that action did not produce an effect corresponding to the quantity of cisplatin which was encapsulated reaching the tumor. In rats administered CDDP-L, it was discovered that the antitumor effect was not only enhanced by the co-administration of caffeine but also further enhanced when the dosing period of caffeine was increased. *Conclusion:* CDDP-L combined with caffeine can produce better results for osteosarcoma.

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REGULATION OF MULTIPLE MYELOMA CELL DIFFERENTIATION BY BONE-MARROW DERIVED MICROENVIRONMENTAL FACTORS

Ben-Zion Katz

The Hematology Institute, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel

Multiple myeloma is characterized by the malignant growth of immunoglobulin producing plasma cells, predominantly in the bone marrow. The disease, at large, remains confined to the bone marrow throughout its progression, pointing to essential interactions between the malignant plasma cells and the bone marrow microenvironment. We studied the effects of primary human mesenchymal stromal cells, and the extracellular matrix protein fibronectin, on the phenotype of multiple myeloma cells. The co-culture of multiple myeloma cells with mesenchymal stromal cells resulted in significant reduction of the expression of the predominant plasma cell differentiation markers CD38 and CD138, and cell surface immunoglobulin kappa chain, in the malignant plasma cells. Plasma cell markers were found to be differentially regulated by adhesive interactions, and stromal-derived soluble factors. While the down-regulation of CD138 by stromal cells was completely dependent on their adhesive interactions with the multiple myeloma cells, interleukin-6 induced specific down-regulation of CD38. Mesenchymal stromal cells, or their conditioned media, inhibited the growth of multiple myeloma cell line, thereby reducing the overall amounts of secreted light chains. The expression of plasma cells differentiation-associated genes was regulated by adhesive interactions with fibronectin. Analysis of primary multiple myeloma bone marrow samples revealed that the expression of CD138, but not CD38 was affected by adhesive interactions. The *ex vivo* propagation of primary multiple myeloma cells resulted in significant increase in their differentiation markers. Overall, the data indicate that the bone marrow derived microenvironmental factors can revert multiple myeloma cells to a less differentiated phenotype by the combined activities of adhesive interactions and interleukin-6. Thus, the sustained, yet contained outgrowth of multiple myeloma may be tightly regulated by the bone marrow microenvironment.

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EXPLORING THE ROLE OF VAV1, A SIGNAL TRANSDUCER PROTEIN THAT FUNCTIONS ONLY IN HEMATOPOIETIC CELLS, IN HUMAN SOLID MALIGNANCIES

G. Lazer, Y. Idelchuk, L. Pe'er, V. Schapira and S. Katzav

The Hubert H. Humphrey Center for Experimental Medicine and Cancer Research, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel

Aberrant signaling and gene expression is a hallmark of cancer and can occur because of genetic changes in signaling proteins or in proteins that modify their signaling activities. New approaches for the treatment of cancer may become apparent through the identification of the genes involved in malignant transformation. An important example of such genes is Vav1, a cytoplasmic signal transducer protein initially identified as an oncogene. Physiological expression of Vav1 is restricted to the hematopoietic system, where its best-known function is as a GDP/GTP nucleotide exchange factor for Rho/RacGTPases, an activity strictly controlled by tyrosine phosphorylation. Vav1 regulates cytoskeletal rearrangement during activation of hematopoietic cells, as well as activation of JNK, ERK, Ras, NF-κB, and NFAT pathways. It also associates with numerous adapter proteins. Since its discovery, research on Vav1 has cycled between understanding its physiological function in the hematopoietic system and understanding how it is dysregulated as a truncated oncogene in fibroblasts. We have recently shown that wild-type Vav1 is involved in human cancers such as lung, breast, ovarian, neuroblastoma, uveal melanoma and prostate cancer. Stimulation of human lung cancer cells (H358 and H441) with Epidermal Growth Factor (EGF) leads to increased tyrosine phopshorylation of Vav1, suggesting that it participates in signal transduction events in these cells. Depletion of Vav1 by siRNA leads to a dramatic reduction in the ability of the H358 lung cancer cells to form foci in agar and develop tumors in mice, indicating that Vav1 can play a decisive role in transformation of lung cancer cells. It is conceivable that misexpressed Vav1 in tissues other than the hematopoietic system participates in the pathogenesis of cancer by activating pathways for both cellular proliferation and cell survival and/or may contribute to the onset of these tumors.

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MODULATION OF MATURATION AND FUNCTION OF HUMAN DENDRITIC CELLS: A NOVEL MECHANISM OF ANTI-TUMOR EFFECT OF *VISCUM ALBUM*

Srini V. Kaveri

Centre de Recherche des Cordeliers, 15, Rue de l'Ecole de Médecine, 75006 Paris, France

Viscum album (VA) is used in cancer as a complimentary therapy. Despite being used for long time, the mode of action of VA containing therapeutics remains unresolved. Their activity has been attributed to immunomodulatory properties in addition to their cytotoxic properties. Dendritic cells (DCs) are the professional antigen presenting cells (APCs) which are crucial in tumor immunosurveillance and in inducing tumor immunity. The tumor cells have the capacity to evade the surveillance by inactivating the DCs through tumor factors such as VEGF, IL-10 and PGE_2. We examined the aspect that the VA preparations exert an immunomodulatory effect on DCs and favor an effective tumor regression. DCs incubated with VA M Spez and VA Qu Spez at 10 μg/ml showed an enhanced expression of antigen presenting HLA-DR molecule and co-stimulatory CD40, CD80 and CD86 molecules along with an increased production of IL-6, IL-8 and IL-1β. The ability of the DCs treated with VA to induce proliferation of allogeneic CD4+ T-cells further substantiates the immunostimulatory effect of VA preparations. These findings should assist in better clarifying the immunomodulatory characteristics of VA preparations and in designing ameliorated therapeutic strategies.

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TREATMENT OF MALIGNANT TUMORS WITH A COMBINATION OF INTRATUMORAL ^{224}Ra-LOADED WIRES AND CHEMOTHERAPY: RETARDATION OF PRIMARY TUMOR GROWTH AND OF LUNG METASTASES

Y. Keisari[1], T. Cooks[1], G. Horev[1], S. Reitkopf[1], M. Efrati[1], G. Marshak[2], M. Schmidt[3], L. Arazi[3,4] and I. Kelson[3,4]

[1]Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, [2]Davidoff Center, Rabin Medical Center, [3]School of Physics and Astronomy, Sackler Faculty of Exact Sciences, Tel Aviv University, and [4]Althera Medical, Tel Aviv, Israel

Objectives: Highly lethal alpha particle radiation has so far not been used in the treatment of solid tumors because of its short range. We developed a new intratumoral treatment for solid cancer utilizing ^{224}Ra-loaded wires that continually release by recoil short-lived daughter atoms that emit alpha particles. These atoms disperse in the tumor and deliver a lethal dose over a region measuring 3-7 mm in diameter. The method was termed Diffusing Alpha-emitters Radiation Therapy (DART). The present study examines the curative effects of a combination between the ^{224}Ra-loaded wires and anti-tumor chemotherapy, against tumors of various histological types. *Methods:* Mouse and human tumor cells from pancreatic, squamous cell (SCC), prostate, and colon carcinomas, were injected subcutaneously to normal and athymic mice, respectively. Mouse and human tumors, 4-10 mm in diameter were implanted with stainless steel ^{224}Ra-loaded wire(s) (0.3 mm-diameter and 3-5 mm long). Chemotherapeutic agents were administered concurrently only to mice bearing mouse tumors. Animals were monitored for tumor development and survival. Also an *in vitro* set-up was used to assess killing of cancer cells by alpha particles. *Results:* Treatment of mouse SCC with two *i.v.* doses of cisplatin (5 mg/kg) given concomitantly with two ^{224}Ra-loaded wires (11-28 kBq), caused substantial growth arrest of 93%, extended survival from 44 to 87 days, and also reduced metastatic spread to the lungs. Treatment of mouse pancreatic tumors with a combination of one ^{224}Ra wire (13-45 kBq) and Gemcitabine (60 mg/kg) achieved significant reduction in tumor volume relative to any treatment modality alone. ^{224}Ra-loaded wires (15-17 kBq/wire), inhibited tumor growth of colon adenocarcinoma tumors (4-6 mm) by 45%, and even led to complete cure. Injection of 5-FU (75 mg/kg) with the ^{224}Ra wire augmented tumor destruction and growth retardation (35%) compared to ^{224}Ra or 5-FU alone. ^{224}Ra-loaded wires retarded the growth of all human derived tumors implanted in athymic mice. *In vitro* experiments with all tumor cells exposed to alpha particles revealed a dose dependent killing of the tumor cells. The combined treatment with alpha particles and chemotherapy achieved the highest killing rate of tumor cells compared to each cytotoxic component alone. *Conclusion:* ^{224}Ra-loaded wires can effectively destroy mouse and human derived solid malignant tumors, both by direct destruction of tumor cells and by causing damage to the tumor microenvironment, mainly the vasculature. The effect can be further potentiated by a combination with chemotherapy. This combined treatment modality holds significant potential for the treatment of non-resectable human cancer.

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SIGNAL TRANSDUCTION THERAPY OF TUMORS WITH MULTIPLE TARGET KINASE INHIBITORS

György Kéri[1,2], László Őrfi, Zoltán Greff, Zoltán Varga, Bálint Szokol, Zoltán Horváth, István Peták and Axel Ullrich[3]

[1]Vichem Chemie Kutató kft., 1022. Herman Ottó u. 15., Budapest, Hungary;

[2]Semmelweis University, Budapest, Hungary;
[3]Max-Planck Institute of Biochemistry, Martinsried, Germany

Signal transduction therapy has become a leading area of modern drug research aiming to inhibit the pathomechanism based on validated target molecules in cellular signaling. Selective inhibition of these false proliferative signals *via* targeting receptor tyrosine kinases and other signaling enzymes, resulting in the induction of apoptosis by depletion of the "survival factors" is one of the most studied and widely accepted concept of modern chemotherapy. Our Nested Chemical Library™ (NCL) technology is based on a knowledge-base approach where focused libraries of kinase inhibitors around 108 core structures and several hundreds scaffolds are used for hit finding and to generate pharmacophore models. We have established a unique proprietary kinase inhibitory chemistry and have developed nM lead molecules against a series of kinases.

Cancer cells have an ability to resist apoptosis and many tumors are resistant to the apoptosis-inducing effects of radiation and chemotherapy. In order to overcome treatment resistance and develop new drugs that target genes and protein products in the apoptosis pathways we have developed multiple target kinase inhibitors to inhibit certain survival factors and induce synthetic lethality in cancer cells. We perform the discovery and validation of a novel, alternative class of inhibitors that target multiple kinase targets and/or protein-protein interactions along some important survival factors.

We have investigated synthetic lethal interactions for the identification of new and more powerful classes of drug targets. The pro-apoptotic effect of the compounds of Vichem's Chemical Validation Library (CVL) was tested on two isogenic cell lines by sub-G1 flow cytometric apoptosis assay and lead molecules were selected. Our novel "Target Fishing" technology aims for selectivity profiling of lead molecules. We have employed proteomic methods to characterize the cellular targets of kinase inhibitors. In the process of drug discovery selectivity profiling of kinase inhibitors is a very important step, inhibiting more than one relevant target can be advantageous (without inhibiting the 'untouchables' such as insulin receptor kinase). So we perform not only selectivity profiling but also iterative target re-identification of drug candidates during the early drug discovery phase, thus providing tools for pharmacology profiling. Using our kinase inhibitor library for the selected survival factor kinase targets, with biochemical kinase assays we have identified several potent hit and lead compounds and generated joint pharmacophores. With cellular kinase and proliferation/apoptotic assays we selected potent multiple target drug candidate lead molecules.

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LATENT TGF-BETA BINDING PROTEINS (LTBPS) IN TARGETING TGF-BETA ACTION

Jorma Keski-Oja, Marko Hyytiainen, Piia Vehvilainen, Olga Tatti, Anna K. Kantola, Kaisa Lehti, Harald von Melchner and Katri Koli

Department of Pathology, The Haartman Institute, University of Helsinki, Haartmaninkatu 8, 00014 Helsinki, Finland

Extracellular matrix (ECM) is a structural framework for cells and tissues, and provides signals to modulate cell behaviour. TGF-β is targeted into the ECM as latent complexes with its LAP-propeptide and via an LTBP molecule. Dysregulation of TGF-beta activity is associated with almost all forms of cancer. LTBPs-1, -3 and -4 associate with and regulate the availability of TGF-b:s 1-3 in tissues. ECM targeting is essential since the surrounding structures define the susceptibility of the complexes to activation processes. To understand these mechanisms we have analyzed LTBP:SL-TGF-beta incorporation into the ECM of normal, malignant and differentiating cells. Using recombinant LTBP fragments we identified critical domains for this function. All LTBPs got assembled into the ECM, but with varying kinetics. Recombinant N-terminal fragments of LTBPs bound readily to fibroblast ECM whereas the C-terminal domains of bound divergently indicating specificity. In osteogenic differentiation of mesenchymal stem cells we observed shift from LTBP-3 to LTBP-1 in association with increased TGF-beta activity and ECM mineralization. LTBP-4 knockout work revealed its regulatory roles in colorectal carcinomas and lung morphogenesis.

Fibronectin (FN) is central in the matrix targeting of LTBPs in most cell cultures. FN acts as an organizer for the other ECM proteins including LTBPs and fibrillin-1. FN plays a central role in the ECM assembly of LTBP-4. Endogenous LTBP-4 was not targeted into the matrix of FN(–/–) fibroblasts. By exogenous FN the LTBP-4 assembly could be rescued. To characterize the FN binding region of LTBP-4, N-terminally shortened constructs were analyzed for their ECM association by IF-microscopy. When the N-terminal domain (first exon area) of the molecule was deleted ECM deposition of LTBP-4 was reduced and delayed. However, during extended culture minor quantities of LTBP-4 accumulated into the ECM, suggestive of alternative protein-protein interactions. LTBP-4 has a C-terminal ECM binding site. This region binds fibrillin-1 (Fbn-1) directly, suggesting that the observed minor binding could be Fbn-1 dependent. LTBP-4/Fbn-1 double staining profiles support this notion. We identified in LTBP-4 heparin binding sites, which may mediate cell attachment. Current analyses revealed a direct interaction between FN and LTBP-4, which evidently plays divergent roles in ECM assembly. The modulation of structures of LTBPs (protease

sensitivity, splicing) provides numerous alternatives for dysregulated TGF-beta deposition in the pathogenesis of malignant diseases.

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GENETIC ALTERATIONS THAT DISRUPT SUBCELLULAR TRAFFICKING OF THE VHL TUMOR SUPPRESSOR PROMOTE TUMORIGENESIS

Mireille Khacho, Karim Mekhail, Karine Pilon-Larose and Stephen Lee

Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5

The von Hippel-Lindau (VHL) tumor suppressor protein is the substrate recognition component of a Cullin-2-containing E3 ubiquitin ligase that recruits the hypoxia-inducible factor (HIF) for oxygen-dependent degradation. The subcellular trafficking properties of VHL are essential for its ability to mediate efficient degradation of HIF and suppress tumor formation. Interestingly, nuclear export of VHL requires ongoing transcription and is independent of the classical NES/CRM1 nuclear export pathway. Examining this uncharacterized nuclear export pathway led to the identification of a discreet motif, reffered to as TD-NEM (transcription-dependent nuclear export motif), that directs transcription-dependent nuclear export of VHL. TD-NEM is targeted by naturally-occurring mutations associated with renal carcinoma and polycythemia in humans. We have also shown that nuclear export of VHL by TD-NEM is mediated through the translation elongation factor eEF1A, a protein implicated in the nuclear export of tRNA in lower eukaryotes. eEF1A interacts specifically with TD-NEM and disrupting this interaction, by point mutations of key TD-NEM residues or siRNA-mediated knockdown of eEF1A, suppresses nuclear export of VHL. We show that naturally-occurring and cancer-causing mutations targeting key residues of TD-NEM disrupt the tumor suppressor function of VHL by altering its nuclear export dynamics. Such mutations restrain the ability of VHL to efficiently mediate oxygen-dependent degradation of HIF. These results demonstrate that eEF1A/TD-NEM-mediated nuclear export is critical to suppress activation of the HIF oncogenic pathway and highlight the essential role of subcellular trafficking in the tumor suppressor function of VHL.

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DEVELOPMENT AND CHARACTERISATION OF APTAMERS AS INHIBITORS OF VITAL PATHWAYS IN CANCER THERAPY

H. Khan[1], V. Makwana[1], N. Courtenay-Luck[2] and S. Missailidis[1]

[1]Department of Chemistry and Analytical Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA; [2]Antisoma Research Ltd, Welwyn Garden City, UK

Aptamers can be described as short oligonucleotides that harbour great potential in their versatile applications, due to their high affinity and specificity for their target of choice. Despite their infancy, aptamers have already emerged at the forefront of clinical research, offering significant contribution as therapeutic agents for a range of diseases. Thus, our research is aimed towards generating aptamer(s) for a specific cancer biomarker that, when blocked, can lead to specific cell kill and thus offer a novel therapeutic potential against cancer.

Aptamers are traditionally selected using SELEX technology, which we have adapted to generate high affinity aptamers on a faster timescale. Aptamer selection experiments against a synthetic peptide of our tumour marker have led to the selection of 3 aptamer species, which appeared in two independent variations of the selection method.

Lack of an initial positive control, in the form or an Ab or other ligand for our target, and limitations in the physical properties our host-ligand system resulted in limitations with regards to characterising the interactions between the aptamer and its peptide target. To overcome such issues, we have synthesised a DNA intercalating dye with different luminescence properties depending on its physical environment. The utilisation of this dye in a fluorescence displacement assay has demonstrated that this technique can provide a suitable means to characterise aptamer-peptide interactions, without the need for modification. However, the ability of the peptide to displace the dye from the aptamer appeared to be dependent on the buffer system used in the assay. CD experiments have verified that this is ultimately due to ligand-induced conformational changes of the aptamer structure, influenced by the buffer system employed. Other techniques used to study the interaction of our biomolecules consist of FRET assays, EMSAs and biacore. Stability assays have demonstrated that the aptamers show remarkable resistance towards nucleases present in human serum and reasonable stability in mouse serum. Flow cytometry experiments further demonstrated that our aptamers are able to bind to cancer cell lines proposed to express our target biomarker, offering a viable tumour marker validation assay. Remarkably, shorter variations of each aptamer, designed on the basis of computational predictions, showed better binding to the cancer cells than their full length counterparts. Finally, our aptamers have shown the ability to effect direct cell kill in prostate and breast cancer cell lines by the proposed inhibition of vital cellular pathways leading to cell apoptosis. Our studies show promising results for these aptamers as cancer

therapeutics and as such these aptamers are now in pre-clinical studies.

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MECHANISM OF ACTION AND DURATION OF TRANSLATION OF THE THYMIDINE KINASE GENE IN ADENOVIRUS-MEDIATED GENE THERAPY OF OVARIAN CANCER

D.G. Kieback[1,2], A.Romano[2], B.Delvoux[2], S.Ollig[1] and D.C. Fischer[2]

[1]HELIOS Medical Center, Aue, Gartenstrasse 6, 08280 Aue, Germany;
[2]GROW Research Institute, University of Maastricht, the Netherlands

Adenovirus (ADV)-mediated gene therapy with the thymidine kinase (TK) Gene under control of the Rous sarcoma virus (RSV) promotor followed by the administration of acyclovir has been established *in vitro* for the treatment of ovarian cancer cells and has been used as the basis for intraperitoneal phase I clinical trials.The purpose of this investigation was to clarify whether cell death after ADV-RSV-TK gene therapy and acyclovir administration is indeed due to apoptosis induction.

It was tested if the synergistic effect of ADV-RSV-TK gene therapy with chemotherapy was limited to the primary mechanism of action or whether the vector transduction itself exerted any pro-apoptotic effect. It is unclear how long a significant degree of transgene translation can be expected after adenovirus-mediated TK transduction, where the transcriptional complex is localized in the nucleus in an episomal fashion and thus without stable integration. The possible interaction of Acyclovir pretreatment with subsequent ADV-RSV-TK transduction also remains to be elucidated.

The epithelial cell lines OVCAR-3 and MDAH-2774 were established from human poorly differentiated serous ovarian cancer.Fluorimetric assay of caspase-3 activity was performed as well as ELISA of the CK 18 split product M30. PARP cleavage was analysed by Western blotting. Transgene expression and cell killing efficacy were analysed based on multiplicity of infection (MOI) and MTT assay.Anti-TK-antibody 1397 was used for immunocytochemistry and Western Blot analysis of TK expression. After transduction with ADV-RSV-TK at an MOI of 66, TK translation increased strongly in MDH 2774 and OVCAR-3 cell lines during the initial 48 hours. Apoptosis induction was established in this investigation as the mechanism of the ADV-RSV-TK gene therapy effect of acyclovir administration by caspase activity and subsequent CK 18 cleavage. Neither acyclovir nor vector administration alone showed any apoptotic activity. The synergistic effect of TK gene therapy and chemotherapeutic agents was shown to be TK induced. Significant anti-PARP 1 activity was found to be an ADV-RSV-TK treatment effect after acyclovir addition. CAR expression was observed on the membranes but also intracellular translocation of CAR takes place dependent on cellular growth patterns TK gene expression is dependent on multiplicity of infection (MOI) and thus on vector dose in a linear fashion. Neither TK expression nor ADV transduction influence CAR expression. Differential susceptibility of different cell lines to TK induced cell killing by Acyclovir metabolites was observed.

CAR expression appears not to be influenced by adenoviral transduction or by the accumulation of the tymidine kinase gene product. Differences in therapeutic sensitivity are most likely mediated by intracellular mechanisms and not by modulation of CAR expression.Virtually constant expression of the TK transgene was observed by Western blot during eight days. Cell killing efficacy was increased by repeated daily administrations of acyclovir. Pretreatment with acyclovir did not result in significantly increased cell killing efficacy. No negative effect of acyclovir on ADV-RSV-TK transduction was observed. The at least week-long expression of the TK transgene with persistently increasing efficacy of cell killing after adenovirus-mediated tumor cell transduction provide a realistic basis for the development of multicycle ADV-mediated TK gene therapy approaches in the treatment of ovarian cancer. Continuous *i.v.* acyclovir treatment or daily oral acyclovir-prodrug therapy might simplify the substrate regimen for the TK gene. Due to its anti-PARP activity it may in the future play a role specifically in the treatment of BRCA1- and 2- related familial ovarian cancer.

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TECHNOLOGICAL ADVANCES IN OVARIAN CANCER SURGERY

D.G. Kieback, A. Süsse and S. Ollig

HELIOS Medical Center Aue, Gartenstrasse 6, 08280 Aue, Germany

Most tumors with intraperitoneal dissemination are not amenable to surgical treatment strategies. In ovarian cancer, however, patient prognosis can be dramatically improved by radical surgical removal of all macroscopic disease at the time of primary surgery. This is necessary in the 75% of patients presenting with advanced disease at the time of primary diagnosis. In order to achieve this goal, multiorgan resections, peritoneal resection and pelvic and paraaortic lymphadenectomy are in many instances warranted in addition to the routinely performed hysterectomy with bilateral salpingo-oophorectomy and omentectomy. In view of the long duration of these surgical interventions of six hours and more, it is of great importance to optimise the rapidity of each surgical step while minimizing blood loss.

To this end, high frequency surgery using electrical energy (HF-Surgery) has recently made major inroads. Energy adjustment directly from the handpiece without need for external help in adjusting generators, computerized energy dosing with vessel sealing and tissue separation by integrated cutting devices have become available. Ultrasound surgery (CUSA) is used in tumor spread to vulnerable surfaces that are otherwise surgically inaccessible or in diffuse coating of large parts of the intestine. In combination with advanced stapling techniques for bowel reconstruction and formation of organ replacement such as continent conduits these options make possible a vastly increased degree of radicality with less complications.

From September 2006 until September 2008 49 patients with ovarian cancer stage III and IV underwent surgical exploration for ovarian cancer, 37 with newly diagnosed disease and 12 with recurrence after more than one year after completion of primary treatment. Complete radical debulking surgery to no macroscopic residual was possible in 46 patients overall, 35 in primary surgery and 11 in recurrent tumors. 3 patients could not be significantly debulked. 1 had extensive infiltration of the entire muscular anterior abdominal wall, 2 suffered from serious comorbidities that made longer interventions impossible. Those underwent primary chemotherapy.

There was no operative mortality. The complete debulking rate of 94% even in this mixed series is in part due to the still relatively small numbers. Our serious, however, confirms data by Eisenkop and colleagues while comparing favourably with European data *i.e.* from Germany, where optimal surgical results are obtained approximately 20% of these cases even in cooperative study groups.

We conclude, that with adequate surgical training and optimal utilization of the technological advances made in surgical instrumentation complete debulking surgery can be performed in the vast majority of patients with advanced ovarian cancer.

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CYTOTOXICITY AND CELLULAR UPTAKE OF DOXORUBICIN AND ITS FORMAMIDINE DERIVATIVES IN HL60 SENSITIVE AND RESISTANT CELLS

Krzysztof Kik[1], Małgorzata Wasowska-Lukawska[2], Irena Oszczapowicz[2] and Leszek Szmigiero[1]

[1]Department of Molecular Pharmacology, Medical University of Lodz, 6/8 Mazowiecka St., 92-215 Lodz; [2]Institute of Biotechnology and Antibiotics, 5 Staroscinska St., 02-516 Warsaw, Poland

Background: In this work we compared cytotoxicity and cellular uptake of doxorubicin (DOX) and its two derivatives, in which the amino group at 3' position was replaced by 2 different formamidino groups (-N=CH-N), where one of the nitrogen atom is a part of morpholine (derivative DOHM) or hexamethyleneimine (DOXH) ring. All tests were performed in HL60 sensitive and HL60/VINC resistant cells. HL-60/VINC cells retain sensitivity toward vincristine, a drug which is typically associated with the classical multidrug-resistance phenotype. Their resistance to anthracyclines is due to the lowered activity of DNA topoisomerase II. *Results:* Cytotoxic activity of DOX toward resistant cells was ~200 times lower when compared with the sensitive HL60 cell line (resistance index 200). Formamidine derivatives of DOX also exhibited decreased cytotoxicity toward HL60/VINC cells but the resistance index for both compounds was ~2 and these compounds were more than 20 times more active in resistant cells than parent drug DOX. It was found that the uptake of DOX was lowered in resistant cells by 16%. The uptake of DOXM and DOXH was also lowered by 26% and 19%, respectively. Thus the changes in the cellular uptake of anthracyclines are not associated with the loss of their cytotoxicity and the fact that cytotoxicity of DOXM and DOXH exceeded the cytotoxicity of DOX. These results suggests that DOXM and DOXH are much more cytotoxic then DOX against HL60/VINC cells because the mechanism of their cytotoxic action is different from that of DOX. This suggestion was proven by the experiments in a cell-free system containing human topoisomerase II and supercoiled pBR322 DNA. DOX strongly stimulated DNA cleavage, whereas its derivatives were inactive. *Conclusion:* It is concluded that the introduction of formamidine moiety containing morpholine or hexamethyleneimine ring is promising way to obtain anthracyclines cytotoxic against tumors cells with altered topoisomerase II activity.

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TUMOR FORMATION DUE TO ABNORMALITIES IN THE β-CATENIN-INDEPENDENT PATHWAY OF WNT SIGNALING

Akira Kikuchi, Hideki Yamamoto and Manabu Kurayoshi

Department of Biochemistry, School of Biomedical Sciences, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima 734-8551, Japan

Wnt signaling is a complex pathway in which β-catenin is viewed as a central mediator in regulating cell proliferation and differentiation. The significance of Wnt signaling in human cancer has been elucidated by the identification of mutations in genes coding for the β-catenin-dependent pathway components, APC, β-catenin, and Axin. Evidence has been recently presented on the importance of the β-catenin-independent pathway activated by Wnt signaling. It is likely that this pathway activates several intracellular signaling

systems to regulate cell migration, adhesion, and polarity. The β-catenin-independent pathway has also been shown to be involved in tumor biology. However, the role of the β-catenin-independent pathway is still unclear. Since Wnt5a is a representative ligand that activates the β-catenin-independent pathway, we sought to clarify how Wnt5a is involved in aggressiveness of gastric cancer. Wnt5a was overexpressed in 71 of 237 gastric cancer cases. The positivity of Wnt5a expression was correlated with advanced stages and poor prognosis of gastric cancer. Wnt5a stimulated cell migration and invasion in gastric cancer cells. Wnt5a activated focal adhesion kinase and small GTP-binding protein Rac, both of which are known to play a role in cell migration. Cell migration, membrane ruffling, and turnover of paxillin were suppressed in Wnt5a knockdown cells. Furthermore, anti-Wnt5a antibody suppressed gastric cancer cell migration. These results suggest that Wnt5a stimulates cell migration by regulating focal adhesion complexes and that Wnt5a is not only a prognostic factor but also a good therapeutic target for gastric cancer. In this talk, we review recent developments in both the functions and mechanisms of the β-catenin-independent pathway of Wnt signaling, with an emphasis on its functional contribution to tumor progression.

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GENE EXPRESSION PROFILING: CANONICAL MOLECULAR CHANGES AND CLINICOPATHOLOGICAL FEATURES IN SPORADIC COLORECTAL CANCER

Jin C. Kim[1,2], Seon Y. Kim[1,2], Seon A. Roh[1,2], Dong H. Cho[1,2], Dae D. Kim[1,2], Jeong H. Kim[3] and Yong S. Kim[3]

[1]Departments of Surgery, University of Ulsan College of Medicine and Asan Medical Center, and [2]Laboratory of Cancer Biology and Genetics, Asan Institute for Life Sciences, 388-1 Poongnap-2-Dong Sonagpa-Ku, Seoul 138-736;
[3]Medical Genomics Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Korea

Background: Although various molecular changes have been identified in colorectal cancer, a clear pattern is detected in only 6.6% of these tumors, indicating the need to identify alternative or subordinate pathways involved in colorectal tumorigenesis and tumor growth. *Methods:* Using microarray gene-expression analysis, we therefore assayed patterns of gene expression, relative to canonical molecular changes and clinicopathological features, in 84 sporadic colorectal cancer patients, standardized by tumor location. Subsets of differentially expressed genes were confirmed by real-time RT-PCR. *Results:* The largest number of genes identified as differentially expressed was by tumor location, and the next largest number by lymphovascular or neural invasion of tumor cells and by mismatch repair (MMR) defects. Amongst biological processes, the immune response was significantly implicated in entire molecular changes observed during colorectal tumorigenesis ($p<0.001$). Amongst 47 differentially expressed genes, seven (*PISD, NIBP, BAI2, STOML1, MRPL21, MRPL16*, and *MKKS*) were newly found to correlate with tumorigenesis and tumor growth. Most location-associated molecular changes had distinct effects on gene expression, but the effects of the latter were sometimes contradictory. *Conclusion:* We show that several differentially expressed genes were associated with canonical molecular changes in sporadic colorectal cancer, possibly constituting alternative or subordinate pathways of tumorigenesis. As tumor location was the dominant factor influencing differential gene expression, location-specific analysis may identify location-associated pathways and enhance the accuracy of class prediction.

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CHEMO-RESPONSIVENESS ASSOCIATED WITH CANONICAL MOLECULAR CHANGES IN COLORECTAL CANCER

Jin C. Kim[1,3], Seon A. Roh[1,3], Dong H. Cho[1,3], Sang N. Yoon[1,3], Chang S. Yu[1,3], Tae W. Kim[2], Seon Y. Kim[4] and Yong S. Kim[4]

Departments of [1]Surgery and [2]Internal Medicine, University of Ulsan College of Medicine, Asan Medical Center, and [3]Laboratory of Cancer Biology and Genetics, Asan Institute for Life Sciences and 388-1 Poongnap-2-Dong Songpa-Ku, Seoul 138-736;
[4]Division of Medical Genetics, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Korea

Background: To assess the canonical molecular changes in colorectal tumorigenesis associated with response to chemotherapy, in order to identify candidate markers. *Methods:* In total, 156 patients received adjuvant postoperative fluoropyrimidine-based chemotherapy, and 32 patients received oxaliplatin- or irinotecan-based chemotherapy following palliative surgery or for metastatic or recurrent colorectal tumors. Representative molecular changes in tumor tissues, including *APC*, Wnt, MMR, RAF, TGF-β, BMP, and p53, had been previously determined in 130 patients, with an additional 42 patients included in this analysis. *Results:* Disease-free survival period (mean±SEM) was significantly longer after fluoropyrimidine-based adjuvant chemotherapy in tumors with TGF-β2 expression (42±1.4 *vs*. 21±4.7 months; $p=0.005$) and *D18S46* LOH or MSI (45.7±1.5 *vs*. 40.5±1.4 months; $p=0.048$). In the metastatic settings, the high disease control rate correlated

significantly with wild-type relative to mutant *APC* and intact MMR relative to MMR defects (p=0.013, respectively). Interestingly, specific molecular steps of tumorigenensis were closely associated with particular toxicities. *Conclusion:* A subset of molecular changes occuring during colorectal tumorigenesis showed significant associations with therapeutic responses and toxicities to chemotherapy regimens, suggesting that these changes may be candidate predictors of response to chemotherapy.

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FAK/SRC PATHWAY PLAYS A KEY ROLE IN FARNESIFEROL C INDUCED ANTIANGIOGENIC AND ANTITUMOR ACTIVITIES

Jae-Ho Lee[1], Sun Choi[2], Yoonji Lee[2], Hyo-Jeong Lee[1], Kwang Seok Ahn[1], Hyo-Jung Lee[1], Eun-Ok Lee[1], Seung-Hoon Choi[1], Junxuan Lu[3] and Sung-Hoon Kim[1,3]

[1]Cancer Preventive Material Development Research Center, College of Oriental Medicine, Kyunghee University, Seoul 130-701, Republic of Korea;
[2]College of Pharmacy and National Core Research Center for Cell Signaling and Drug Discovery, Ewha Womans University, Seoul, 120-750, Republic of Korea;
[3]The Hormel Institute, University of Minnesota, 801 16th Avenue NE, Austin, MN 55912, USA

Farnesiferol C (FC) is a natural compound from *Ferula assafoetida* L. that has been used for cancer treatment as a folk remedy; its anti-tumor efficacy and mechanisms are not yet determined. Thus, in the present study, we first examined the anti-angiogenic activities and associated mechanisms of FC using a battery of *in vitro*/*ex vivo* assays. FC exerted cytotoxicity against non-proliferating human umbilical vein endothelial cells (HUVECs) with IC_{50} of ~70 μM. Within the non-cytotoxic ranges of exposure (10~40 μM), FC inhibited vascular endothelial growth factor (VEGF) induced cell proliferation, migration, invasion and tube formation (capillary differentiation) and also the expression of matrix metalloproteinase (MMP) 2 in the VEGF-treated HUVECs. Also, FC decreased the binding of VEGF to VEGFR-1/Flt-1, but not to VEGFR-2/KDR/Flk-1. Interestingly, the dampened endothelial cellular responses were preceded by a rapid inhibitory action (examined within 10 min of VEGF stimulation) of FC on a number of key VEGF-induced signaling pathways: decreased VEGF-induced phosphorylation of Src and FAK without affecting VEGFR-2 autophosphorylation or AKT phosphorylation and decreased the phosphorylation of mitogen activated protein kinases (MAPKs), such as ERK1/2, c-JUN N-terminal kinase (JNK) and p38MAPK. Consistently, molecular modeling studies predicted that FC can inhibit Src kinase activity via its preferential docking to the ATP-binding site of Src kinase rather than VEGFR2. In addition to HUVEC model, FC inhibited the sprouting of VEGF-treated rat aortic endothelial cells in a concentration dependent manner in an *ex vivo* model. Furthermore, FC significantly inhibited the *in vivo* growth of mouse Lewis lung cancer cells inoculated on the flank of syngenic mice at a dose of 1 mg/kg without any negative effect on the body weight of the host mice. Immunohistochemistry also revealed that its anticancer efficacy is associated with decreased expressions of microvessel density (CD34), proliferative index (Ki-67) and Src. Taken together, these findings suggest that Farnesiferol C possesses strong anti-angiogenic potential *via* FAK/Src and MAPK pathways as a cancer chemopreventive or therapeutic agent.

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SHIKONIN-INDUCED CELL CYCLE ARREST AND APOPTOSIS IN HUMAN BREAST CARCINOMA MCF-7 CELLS

Sun-Joong Kim and Hyo Ihl Chang

College of Life Sciences and Biotechnology, Korea University, Seoul 136-701, South Korea

Shikonin isolated from *Lithospermum erythrorhizon* roots has been reported to confer biological properties including antibacterial, wound healing, anti-inflammatory, antithrombotic, and antitumor effects. We investigated, together with the structurally related naphthoquinone, the effects on the induction of apoptotic cell death in the human breast carcinoma cell line, MCF-7. Shikonin was extracted from *Lithospermum erythrorhizon* by sonication with n-hexane followed by hydrolyzed in 1N NaOH, and analyzed by UPLC coupled with TOF-MS/MS. Chromatographic separation was achieved on a reversed-phase Luna C_{18} column and step gradient elution resulted in a total run time of about 25 min and the full scan mass spectra of shikonin. The protonated molecules found for shikonin were m/z 287.12. The cytotoxic potential of shikonin was assessed through MTT assay. Cell-cycle analysis, Reactive Oxygen Species (ROS) and Mitochondrial Membrane Permeability were performed by Flow Cytometry. Treatment with 0-20 ppm of shikonin did not induce significant apoptosis, but rather induced G_0/G_1-phase arrest in MCF-7 cells. Flow cytometric analysis indicated that shikonin directly increased intracellular oxidative stress based on the cell permeable dye, 2',7'-dichlorodihydrofluorescein diacetate(DCFH-DA) acting as an indicator of reactive oxygen species(ROS) generation. Also, shikonin decreased the mitochondrial membrane potential ($\Delta\psi_m$), in MCF-7 cells. Overall, our results demonstrated that shikonin treatment causes cell death by activating pathway inducing G_0/G_1-phase arrest and apoptosis.

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IMMUNOTHERAPY OF LUNG CANCER AND OVERVIEW OF IMMUNOTHERAPY–PAST, PRESENT AND FUTURE

Hideki Kimura

Chiba Cancer Center Japan, Nitona-Cho 666-2, Chu-o-ku Chiba City Japan

Introduction: The efficacy of immunotherapy for cancer patients is still under debate. Although accumulating evidence indicates that the immune system recognizes tumors and mounts responses against cancer, immunotherapy for cancer patients has not gained as much public support as a standard therapy such as surgery, chemotherapy or radiation therapy. In our presentation, we report results of experimental analysis of killer cell and dendritic cell production from regional lymph nodes of primary lung cancer patients. In the presence of dendritic cells obtained from tissue culture of regional lymph nodes of primary lung cancer patients, we obtained activated killer cells which demonstrated specific cytotoxic activity against autologous cancer cells. By adding peripheral blood lymphocytes to long-term tissue culture of regional lymph nodes, we have succeeded in generating specific killer cells sufficient for the immunotherapy of cancer patients. Using these cells as a source of adoptive immunotherapy, we report promising results of a phase II study of post-surgical adjuvant chemo-immunotherapy against lung cancer patients. Furthermore we will discuss future possibilities and limits of immunotherapy. *Patients and Methods:* Pathologically diagnosed N2 lung cancer patients were selected for post-surgical adjuvant chemo-immunotherapy. The activated killer cells and dendritic cells (AKT-DC) obtained from tissue cultures of tumor-draining lymph nodes (TDLN) or from TDLN co-cultured with peripheral blood lymphocytes (TDLN-Pb) were used for the adoptive transfer of immunotherapy. The patients received surgery and 4 courses of chemotherapy along with 10 to 12 courses of adoptive immunotherapy every 2 months for 2 years. *Results:* There were 31 N2 patients eligible for the study. Three cases were excluded because of refusal by the patients after 1-2 courses of immunotherapy. For the 28 cases treated, a total of 313 courses of immunotherapy were administered. The main toxicities were fever (78.0%), chill (83.4%), fatigue (23.0%) and nausea (17.0%) on the day of cell transfer. The 2- and 5-year survival rates were 88.9 %(95.9-81.9; 95% confidence interval, C.I.) and 52.9% (76.4-29.4; C.I.). The 5-year survival rate of the patients with same stage who received chemotherapy and surgery without immunotherapy was 12.5%. *Conclusion:* Adoptive transfer of activated killer cells and dendritic cells from the tumor-draining lymph nodes of primary lung cancer patients is feasible, safe, and give us promising hope for the future prospective study of immunotherapy.

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LOW PENETRANCE GENETIC SUSCEPTIBILITY FACTORS IN HUMAN CARCINOGENESIS

István Kiss[1], Zsuzsanna Orsós[1], Antal Tibold[1], Zsuzsanna Varga[2], József Cseh[3], András Csejtei[4] and István Ember[1]

[1]Institute of Preventive Medicine, Faculty of Medicine, Pécs University of Sciences, Pécs;
[2]Department of Oncotherapy, Pécs University of Sciences, Pécs;
[3]Department of Oncology, Fejér County "Szent György" Hospital, Veszprém;
[4]Department of Oncoradiology, Vas County "Markusovszky" Hospital, Szombathely, Hungary

Cancer is a process caused by gene-environment interactions. The cancer-related genetic factors can be high-penetrance genes, causing hereditary tumors and cancer syndromes, or so called individual susceptibility factors. The latter group typically consists of minor variants – allelic polymorphisms – in genes regulating cell cycle, cell division, DNA repair, apoptosis and metabolism of carcinogenic substances.

The individual susceptibility factors are not strong enough to cause familial aggregation of tumors, but on a population level they can significantly alter the risk of certain cancers. However, in an interaction with each other, or with environmental exposures, the low penetrance susceptibility factors might already cause substantial risk increase in "high-risk" individuals. Genotyping for several allelic polymorphisms might give a possibility for individualized prevention, by giving complex information on personal susceptibility traits.

The lecture gives an overview on the most important categories of low penetrance cancer susceptibility factors, their mode of action and population level significance. Results of our molecular epidemiological studies on colorectal cancer, thyroid cancer and other tumors, concerning the effects of allelic polymorphisms of metabolizing enzymes, tumor suppressor genes and DNA repair genes will also be reviewed, including recent, unpublished works.

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EXOCRINE PANCREATIC CANCER: NEW CONCEPTS, NEW DRUGS, PERSPECTIVES

R. Klapdor

Internal Practice, Centre for clinical and experimental tumour marker diagnosis and therapy (ZeTDT)GmbH, Hamburg, Germany

During the past 15 years most of the scientific clinical studies reported on study protocols following only 1 treatment regimen. The mean survival of 6-8 months in these studies is even nowadays accepted as a reference value for survival of patients suffering from advanced exocrine pancreatic cancer.

Reviewing the literature the main reasons for this disaster seem to be: 1. The known cytostatic drugs only show a limited/disappointing efficacy in the case of single agent therapies. 2. Palliative therapy in general is started too late, not allowing a second or third line therapy. 3. Follow up only based on imaging methods performed in more or less long intervals. 4. Study-protocols to investigate new drugs or drug-combinations mainly base on 1- line- treatment strategies. 5. Symptomatic supportive treatment with all their actual facilities is not employed in a way, possible today (nutrition, pain therapy, endoscopic palliative surgery, palliative resective surgery etc.). 6. Some kind of treatment nihilism due to the known limited efficacy of Gemcitabine and it's combinations during the past 14 years seem to paralyse some activities in the oncological field.

Our own experience however suggests that the outcome of these cancer patients can be improved by earlier diagnosis and earlier start of palliative tumortherapy, by valid follow-up based on imaging methods every 6-8 weeks in combination with tumor marker determinations every 2-4 weeks, by consequent offer of best supportive care to the patients and by treatment concepts following the concept of EOSPC (Efficacy Orientated Sequential Poly-Chemotherapy). Following this concept we were able to report on median survival rates of 16 months for all patients and of 18 months for locally advanced and 13 months for metastasized carcinomas resp.. 73% of our patients allowed to start a second line therapy, 68% also a third line treatment. M1 patients with >1 effective treatment sequence (49% of the M1 tumors diseases) showed a median survival of 18.5 months. As cytotoxic drugs we mainly used Gemcitabine, 5-FU, Folinic acid, Oxaliplatin, Mitomycin-C and/or Irinotecan as single drugs or in combinations. New drugs acting by new mechanisms like *e.g.* Erlotinib, new combinations or local regional approaches seem to further improve the survival.

Summarizing the data support the concept of efficacy orientated sequential pancreatic cancer therapy. Consequently also clinical prospective studies should be planned no longer as 1-line concepts, but at least as 2- or 3- line concepts with new drugs or drug combinations to be evaluated as 1st-, 2nd- or 3rd lines.

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CHRONIC AND BREAK-THROUGH PAIN IN CANCER PATIENTS

U.R. Kleeberg

HOPA, Hamburg, Germany

Although the pathophysiology of pain has been studied in great detail, cancer-related chronic pain syndromes and break-through pain are highly complex and difficult to analyze in individual patients, reflecting the clinical day to day problems with an optimal and tailored treatment. Several surveys on patient satisfaction with the quality of pain control, both for in- as well as in out-patients of specialized oncology units, leave quite some room for improvement. Roughly 20% of cancer patients continue to suffer from pain, though improved, despite optimal interdisciplinary efforts.

The dominant clinical problems stem from the delicate balance between pain control, treatment-related side-effects and patient adherence. Increasing dosage and dose intensity of Opiods, combinations with adjuvants and psychosocial support may help in most situations, but definitely not in all, especially in neuropathic and break-through cancer pain (BTCP).

New insights in the pathogenesis of pain and new drugs and drug combinations have moved us on a little further, still being unsatisfactory though in difficult clinical situations. Teaching propaedeutics in Palliative Care remains one of the dominant tasks in academic medicine: Detailed case history, differential diagnosis, documentation of the course, and repeated consultation of all disease-related disciplines under the guidance of the medical oncologist will ameliorate the cancer patient's suffering and allow for new strength "zum Menschsein".

An overview about the current state of the art will be given.

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THE ROLE OF MICROENVIRONMENT IN EBV ASSOCIATED LYMPHOID MALIGNANCIES

Eva Klein

Department of Microbiology, Tumor and Cell Biology. Karolinska Institute, 17177, Stockholm, Sweden

Depending on the differentiation and maturation of EBV-genome carrying cells, virally encoded proteins are expressed in various combinations. These proteins determine the fate of the viral genome harbouring cells. Virus transformed B lymphocytes - lymphoblastoid cell lines - LCL- express six virally encoded nuclear (EBNA) and three surface localised (LMP) proteins. This phenotype is encountered only in B lymphocytes and it is associated with cell proliferation. Therefore, it is usually referred to as growth transformation program and also as Type III EBV expression. Such cells are readily recognized by the immune response. Their proliferation therefore is inhibited in healthy individuals. Consequently in immunosuppressed patients this mechanism does not function and the risk for EBV associated B cell malignancies is high. In other cell types which carry the EBV genome the expression of the virally encoded proteins is restricted, such as in NK cells, T-cells.

They express only EBNA-1 and LMP-1, thus they lack the nuclear protein EBNA-2 that is required for the proliferating inducing capacity of the virus. In such cells the presence of the viral genome does not induce proliferation. However it may affect the phenotype of the cell and alter their behaviour, by avoidance of apoptosis, by inducing enrichment of inflammatory cells in their surrounding and/or their response to growth inducing intercellular contacts or to cytokines may be intensified. There is evidence for such mechanisms in EBV associated Hodgkin's lymphoma and nasal NK lymphoma.

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CELLULAR SENESCENCE AND CANCER DEVELOPMENT

Dimitris Kletsas

Laboratory of Cell Proliferation and Ageing, Institute of Biology, National Centre for Scientific Research "Demokritos", 15310 Athens, Greece

The majority of normal somatic cells cannot proliferate indefinitely and after a limited number of duplications they enter a state called replicative senescence, characterized mainly by the cell's inability to proliferate. This phenomenon is the consequence of a progressive telomere shortening, that is perceived by the cells as a DNA damage, leading to the activation of p53 tumor suppressor and thus to cell cycle arrest. In addition, the cells can senesce prematurely after exposure to various types of genotoxic stress. Interestingly, the overexpression of several oncogenes in normal cells leads also to premature senescence, indicating that senescence is a potent anticancer mechanism. We have recently shown that this oncogene-induced senescence is also the outcome of a DNA damage response, suggesting common mechanisms underlying replicative and stress-induced senescence. Finally, several DNA damaging agent provoke a senescent-like phenotype in tumor cells.

Beyond their inability to proliferate, senescent cells express a pro-inflammatory phenotype that is believed to contribute to the ageing process and to the development of age-related disorders. We have shown that p53 is responsible for the senescence-associated overexpression of ICAM-1, a crucial pro-inflammatory molecule, indicating that the various features of senescence (cell cycle arrest and the pro-inflammatory phenotype) are, at least in part, linked with the same molecular mechanisms.

Although cellular senescence is considered an anticancer mechanism, it has been proposed that senescent cells, due to their specific phenotype create a permissive environment for the growth of cancer cells, supporting the idea that the senescence response is antagonistically pleiotropic. In this vein, we have shown that subcytotoxic doses of ionizing radiation, used in several anticancer treatment regimes, provoke premature senescence in stromal fibroblasts, *via* a DNA damage response, and these senescent cells enhance the growth of cancer cells, both *in vitro* and in immunocompromised mice *in vivo*. The mechanisms underlying this phenomenon will be discussed. These findings support the idea that replicative- or stress-induced- senescence may play a role in tumorigenesis late in life.

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THE NUCLEOTIDE EXCISION REPAIR COMPLEX ERCC1-XPF AS A POSSIBLE FACTOR FOR REPAIR DEFICIENCY AND CISPLATIN SENSITIVITY IN TESTIS TUMOR CELLS

S. Usanova, A. Piée-Staffa, U. Sied, B. Kaina and B. Köberle

Institute of Toxicology, University of Mainz, Obere Zahlbacher Strasse 67, 55131 Mainz, Germany

Metastatic testicular germ cell tumors (TGCT) are cured in over 75% of patients using cisplatin-based combination therapy. The reasons underlying this cisplatin sensitivity are not yet known. Cell lines derived from TGCTs retain this cisplatin hypersensitivity *in vitro*, therefore providing a good model system for investigating the factors controlling cisplatin sensitivity. Our earlier data showed that testis tumor cells have a reduced capacity to repair cisplatin-induced DNA damage, suggesting that repair deficiency might be a factor for the observed cisplatin sensitivity. In further studies, we found that the nucleotide excision repair (NER) factor ERCC1-XPF is reduced in testis tumor-cell lines, indicating a possible role of ERCC1-XPF for repair deficiency and cisplatin sensitivity of testis tumor cells. Cisplatin induces both intra-strand adducts (IA) and inter-strand crosslinks (ICLs). To investigate repair of IAs, we used the method of DNA slot blotting, while repair of ICLs was investigated using the Comet assay. We found that the repair of IAs was slightly reduced in the two testis tumor cell lines compared to a cisplatin-resistant bladder cancer cell line. Repair of ICLs, however, was significantly reduced in the testis tumor cells compared to the bladder cancer cell line. To analyse the causal role of ERCC1-XPF for repair deficiency and cisplatin sensitivity in testis tumor cells, we overexpressed ERCC1-XPF in the testis tumor cell lines 833K and SuSa using a mammalian expression vector. Overexpression of ERCC1-XPF increased ICL repair in 833K cells, suggesting that ERCC1-XPF might be rate-limiting for repair of ICLs in testis tumor cells. Investigations into the effect of ERCC1-XPF overexpression on cisplatin sensitivity in testis tumor cells are currently under way.

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INVESTIGATION OF THE HERITABILITY OF THE CANCER RESISTANT PHENOTYPE OF THE SR/CR MOUSE

Janne Koch[1], Anna Boschian[1], Jann Hau[1] and Klaus Rieneck[1,2]

[1]Department of Experimental Medicine, University of Copenhagen and University Hospital, 3 Blegdamsvej, 2200 Copenhagen N;
[2]Department of Clinical Immunology, H:S Blood Bank, 2034, Copenhagen University Hospital, Blegdamsvej 9, DK-2100 Ø Copenhagen, Denmark

The SR/CR mouse model of cancer resistance was discovered in 1999. It is resistant to a number of different cancer cell lines and the heritable phenotype was demonstrated on different genetic backgrounds. The cancer resistance is transferable to other strains of mice by adoptive transfer of innate immune cells. We independently, for the first time, confirm the findings of the SR/CR phenotype of cancer resistance to the S180 cell line in mice of two different genetic backgrounds: BALB/c and C57BL/6. The SR/CR mice were screened by intra peritoneal injection of S180 cells. The frequency of the SR/CR phenotype in the present study was 30% for the BALB/c strain and 22% for the C57BL/6 strain in the first litters, but the overall frequency was 8% for both strains. A frequency of about 30% was reported in the original US-colony. A litter seriation effect on the frequency of the SR/CR phenotype was recorded. The phenotype frequency in the first born litters was similar to the one recorded in the founder colony in the US. There was no significant difference in the frequency of the SR/CR phenotype between the two genders, but the overall frequency of the SR/CR phenotype was significantly higher in litters from SR/CR mice on BALB/c background compared to litters from SR/CR mice on C57BL/6 background.

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REDUCTION OF CHEMOTHERAPY-INDUCED NAUSEA AND VOMITING (CINV) THROUGH TREATMENT WITH CLONAZEPAM

Minako Koga[1,2], Momoe Nakadozono[3], Kazutaka Nukariya[2], Hiroko Nogi[4], Tadashi Kobayashi[5], Yusuke Ochiai[2] and Kazuhiko Nakayama[2]

[1]Department of Psychiatry, Machida Municipal Hospital, 2-15-41 Asahi-machi, Machida-shi, Tokyo, 194-0023, Japan; Department of [2]Psychiatry, [3]Nursing, [4]Surgery and 5Clinical Oncology, The Jikei University School of Medicine, 3-25-8 Nishi-shimbashi, Minato-ku, Tokyo 105-8461, Japan

Objective: To report on how the prevention of chemotherapy-induced nausea and vomiting (CINV) has been successfully achieved by administering clonazepam. *Methods:* We identified a patient who had recovered from CINV after taking clonazepam. Based on the outcome of this case, we conducted an investigation into the cases of 26 patients who still experienced CINV despite taking 5HT3 and corticosteroids. *Results:* Significant differences were found in following symptoms nausea, vomiting and appetite loss, and the rates of partial remission and complete remission of the three symptoms were as follows: 76%/47%, 93%/79% and 72%/50%, respectively. *Conclusion:* Clonazepam may be useful in the control of CINV. We believe that clonazepam contributed to the outcome in the following ways: (i) anxiolytic effect, (ii) anticonvulsant effect on myoclonus.

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THE ROLE OF ENDO180 IN THE DEVELOPMENT OF METASTATIC PROSTATE CANCER BONE LESIONS

Giolanta Kogianni[1], Kyriakos Elefteriou[2], Jonathan Waxman[1], Justin P Cobb[2] and Justin Sturge[1]

[1]Prostate Cancer Research Group, Department of Oncology, Division of SORA, Imperial College London, Hammersmith Hospital Campus, London;
[2]Department of Orthopaedic Surgery, Division of SORA, Imperial College London Charing Cross Hospital Campus, London, UK

Background: Metastasis to bone is a major clinical complication in patients with advanced prostate cancer (Pca) and can cause either bone forming or bone degrading lesions. Evidence from recent *in vivo* studies has identified an important regulatory function for the recycling collagen-binding and internalisation receptor Endo180 in bone development. Here we are investigating whether expression of this collagen receptor can be regulated in prostate bone lesions. *Materials and Methods:* Tissue biopsies from human prostate bone metastases were analysed by co-staining for Endo180 with stromal (vimentin) and epithelial (pan-cytokeratin) markers and immunofluorescence microscopy. Co-culture of human primary bone osteoblasts (hOBs) and prostate cells (PCs) derived from benign hyperplasias, primary tumours and various metastatic lesions were characterised for temporal changes in mineral deposition, alkaline phosphatase activity, collagen turnover and Endo180 expression induced by either direct hOB-PC interaction or soluble factors released into their conditioned media (CM). Quantification of Endo180 expression was assessed by Western blot and immunofluorescent staining analysis. Local ethical approval was given for the use of human tissues in this study. *Results:* Endo180 was strongly expressed by both osteoblastic/osteocytic and prostate cancer cells located in metastatic bone lesions. Levels of alkaline phosphatase activity

and mineral deposition by OBs were reduced, whereas collagen production was increased by direct hOB-PC interaction and soluble factors released by PCs with an invasive phenotype. The constitutive expression of Endo180 by hOBs was unaltered in co-cultures with all of the PCs tested. Direct hOB-PC interaction, but not hOB CM, resulted in the up-regulation of Endo180 expression on PCs with an invasive phenotype. *Conclusion:* Endo180 is strongly expressed in prostate bone metastases where its up-regulation in prostate cells is mediated by their direct interaction with osteoblasts. These findings suggest that the constitutively recycling collagen receptor Endo180 could play an important role during active collagen remodelling associated with the pathology of these lesions.

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ENDO180 EXPRESSION BY TUMOUR CELLS WITH AN INVASIVE PHENOTYPE CORRELATES WITH PROSTATE CANCER PROGRESSION

Giolanta Kogianni, Marjorie M. Walker, Jonathan Waxman and Justin Sturge

Imperial College London, London, UK

Purpose: In our previous work, Endo180 was found to have prognostic value for invasive basal breast carcinomas. Here we investigated Endo180 expression in prostate cancer progression with its co-functional partners in tissue collagen remodeling and cell migration: membrane type-1 matrix metalloproteinase (MT1-MMP); urokinase-type plasminogen activator receptor (uPAR); and urokinase-type plasminogen activator (uPA). *Materials and Methods:* Tissue microarray (TMA) containing 169 prostate tissue samples clinically graded as benign prostate hyperplasia (BPH) or Gleason score 6-10 was analysed by immunofluorescent co-staining of Endo180, pan-cytokeratin (pCk) and complete quantification of % total stromal (pCk^-) and epithelial (pCk^+) cells in all tissue cores. Co-expression of Endo180 with vimentin, MT1-MMP and uPAR-uPA was also investigated. Significant differences and correlations between categorical variables, including clinical grade and serum prostate-specific antigen (PSA), were determined using two-sided Tukey test, Pearson correlation and linear regression. *Results:* Increased % total $Endo180^+/pCk^-$ and $Endo180^+/pCk^+$ cells confirmed stromal and epithelial up-regulation of Endo180 in all Gleason grades compared to BPH. Epithelial Endo180 expression displayed strong positive correlation with Gleason grade for which it was a stronger predictor than serum PSA. Co-staining with vimentin, MT1-MMP, or uPAR-uPA by invasive tumor and reactive stromal cells revealed differential patterns of expression during disease progression. *Conclusion:* Endo180 is a reliable predictor of clinical grade, supporting its use as a biomarker for the diagnosis of prostate cancer. The potential molecular interplay of Endo180 with MT1-MMP and uPAR-uPA be at distinct stages of prostate cancer progression provide potential new pathways to targeted in the prevention of metastasis.

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PIK3CA AMPLIFICATION IS ASSOCIATD WITH RESISTANCE TO CHEMOTHERAPY IN OVARIAN CANCER PATIENTS

Iwona K. Kolasa[1], Izabela Ziolkowska-Seta[2], Magdalena Murawska[3], Joanna Moes[1], Agnieszka Timorek[4], Agnieszka Dansonka-Mieszkowska[1] and Jolanta Kupryjanczyk[1]

[1]Department of Molecular Pathology, The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Roentgena 5, 02-781 Warsaw;
[2]Department of Gynecologic Oncology, The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Roentgena 5, 02-781 Warsaw;
[3]Department of Biostatistics, The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Roentgena 5, 02-781 Warsaw;
[4]Department of Obstetrics and Gynecology, Brodnowski Hospital and II-nd Faculty of Medicine, Medical University, Kondratowicza 8, 03-242 Warsaw, Poland

The PI3K/AKT signaling pathway controls important cellular processes such as cell proliferation and apoptosis. The *PIK3CA* gene encoding the catalytic subunit of the PI3K is mutated and/or amplified in various neoplasms, including ovarian cancer. We aimed to evaluate *PIK3CA* alterations and their clinical importance in ovarian cancer patients.

Molecular analysis was performed on 117 ovarian carcinomas with the use of qPCR, SSCP and sequencing. In a group of 98 patients with complete clinical data, 62 patients were treated with standard taxane-platinum regimens and 36 patients with platinum-cyclophosphamide regimens. A multivariate analysis was performed by Cox and logistic regression models.

PIK3CA mutations occurred in 5/117 (4.3%) carcinomas, exclusively in the endometrioid and clear cell types (p=0.0002); they were also associated with low FIGO stage (p=0.0003), low tumor grade (p=0.045) and early patient age at diagnosis (p=0.0005). *PIK3CA* amplification (predominantly low-level) was found in 28/117 (24%) ovarian carcinomas and was more frequent in *TP53* mutant tumors (p=0.012). *PIK3CA* amplification strongly diminished odds of complete remission (p=0.033, OR=0.25) and platinum sensitive response (PS, p=0.004, OR=0.12) in the taxane-platinum treated patients. The odds of PS were also much lower in all patients with the *PIK3CA* amplification evaluated together, regardless of the treatment applied (p=0.001, OR=0.18).

Our results suggest that *PIK3CA* amplification may be a marker predicting ovarian cancer response to chemotherapy.

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ARRAY-COMPARATIVE GENOMIC HYBRIDISATION: A NEW TOOL IN CANCER DIAGNOSIS AND RESEARCH

Aggeliki Kolialexi

Department of Medical Genetics, Athens University School of Medicine, Athens, Greece

Genetic alterations are key features of cancer and typically target biological processes and pathways involved in cancer pathogenesis. DNA copy number changes are common in cancer and lead to altered expression and function of genes residing within the affected region of the genome. Array-comparative genomic hybridisation (a-CGH) is a comprehensive, genome-wide screening procedure for detecting DNA copy number imbalances which can be rapid, less labour-intensive than karyotype banding analysis and highly amenable to automation. a-CGH has provided new information on copy number changes in cancer on a genome-wide level and has doubled the detection rate of pathogenic chromosomal imbalances in patients. A number of new regions frequently involved in gains and losses have been identified and data obtained have been utilized in cancer classification. This has been possible by increasing the resolution level from the 5 Mb obtained using conventional cytogenetic techniques to as low as 100 kb by array technology. More importantly, aCGH analysis has allowed accurate localization of specific genetic alterations associated with tumor progression, therapy response, or disease outcome. Understanding cancer, however, will require profiling of transcriptome, miRNAome, epigenome, and proteome to fully comprehend complex tumor behavior.

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ON THE TRAIL TO A CURE ? – APOPTOSIS *VIA* DEATH RECEPTORS IN EWING'S SARCOMA

Udo Kontny

Division of Pediatric Hematology and Oncology, Center for Pediatrics and Adolescent Medicine, University Medical Center Freiburg, Freiburg, Germany

Ewing's sarcoma is the second most common malignant bone tumor in children and adolescents. Tumors arise from mesenchymal stem cells and are characterized by fusion of EWS with a member of the ETS family of transcription factors. About 30% of patients present with metastatic disease. For these, as well as for patients with recurrent disease, survival rates are <20%. In order to identify new targets for therapy, we have analyzed the extrinsic apoptotic pathway in Ewing's sarcoma. Whereas the majority of tumor cells bear receptors for FasL and TRAIL, only the latter pathway proves to be intact in most tumor cells. *In vitro*, about 80% of cell lines are susceptible to TRAIL-mediated apoptosis. In TRAIL-resistant cell lines absence of caspase-8 expression has been identified as a cause of resistance. Deficient expression of caspase-8 has also been observed in about 25% of Ewing's tumor specimens. Resistance to TRAIL-mediated apoptosis can be overcome by incubating caspase-8-deficient cells with interferon-gamma at concentrations achievable in humans. TRAIL has also been shown to be active against Ewing's tumors *in vivo*, in a mouse xenograft model. Whereas in this model the combination of TRAIL and interferon-gamma did not increase suppression of tumor growth in primary tumors when compared to TRAIL alone, it was significantly more active in preventing the occurrence of metastases. Based on these findings, the combined application of TRAIL and interferon-gamma makes it an attractive candidate for evaluation in clinical trials in patients with Ewing's sarcoma.

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IMPACT OF IFN-γ GENE ON THE RISK OF CERVICAL CANCER

D.M. Kordi Tamandani[1], R.C. Sobti[2], M.Shekari[2], M. Mukesh[2] and V. Suri[3]

[1]Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran
[2]Department of Biotechnology, Panjab University, Chandigrah, India.
[3]Department of Obstetrics and Gynaecology Post Graduate Institute of Medical Education and Research, Chandigrah, India

Background: Cervical cancer, the second most common malignancy in women worldwide, is almost invariably associated with infection by human papillomavirus (HPV). However, although many women are infected with high-risk types of HPV, only a subset of infected women will ever develop cervical cancer. Therefore, host genetic factor may play a role in cervical carcinogenesis. Several studies suggested that immunological components thus play a key role in the development of cervical cancer. Interferon gamma (IFN-γ) is a cytokine produced by activated T-cells and natural killer (NK) cells that enhances cellular immune responses by increasing T-cell cytotoxicity and NK-cell activity. *Objectives:* The striking correspondence between the various biological activities of IFN-γ and the immunological modifications observed in cervical carcinomas, prompted us to study single nucleotide polymorphism (SNP), T to A, located at the +874 position and measure *IFN-γ* messenger RNA (mRNA) at the tumor site. *Methods:* DNA was isolated from peripheral blood

of 200 patients with cervical cancer and 200 healthy controls. The allele polymorphism at position +874 in the *IFN-γ* gene was studied by ARMS-PCR (Amplification Refractory Mutation System) and *IFN-γ* messenger RNA (mRNA) in the tumor was measured by means of a semi-quantitative polymerase chain reaction (PCR) assay. Variation in the promoter region of *IFN-γ* gene, was investigated through sequencing. *Results:* Genotypes *AT* and *AA+AT* increased the risk of cervical cancer (OR=3.3, 95% CI 2.05-5.2, p=0.0000002; OR=2.9, 95% CI 1.9-4.6, p=0.0000007), respectively. Thus the semi-quantitative analysis reflected the similar level of mRNA expression of *IFN-γ* gene in patients suffering from cervical carcinoma to that in healthy control. *Conclusion:* This is the first study to provide evidence for the effect of *IFN-γ* on the risk of cervical cancer in North Indian population.

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CLINICOPATHOLOGICAL FEATURES AND PROGNOSTIC FACTORS OF EPSTEIN-BARR VIRUS-ASSOCIATED GASTRIC CARCINOMA

Chihaya Koriyama[1], Suminori Akiba[1], Michiyo Higashi[2], Francia Campos[1], Kazunobu Sueyoshi[3], Suguru Yonezawa[2] and Yoshito Eizuru[4]

Departments of [1]Epidemiology and Preventive Medicine, [2]Human Pathology, and [4]Division of Oncogenic and Persistent Viruses, Center for Chronic Viral Diseases, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima, 890-8544, Japan
[3]Department of Pathology, Kagoshima City Hospital, 20-17 Kajiya-cho, Kagoshima, 892-8580, Japan

Epstein-Barr virus (EBV) is a human carcinogenic virus and is known to cause Burkitt's lymphoma, undifferentiated nasopharyngeal carcinoma, Hodgkin's disease and some types of lymphomas. Its involvement was also found some of gastric adenocarcinoma cases, and about 10% of gastric carcinomas harbor clonal EBV (EBV-GC). Although LMP1, an important EBV oncoprotein, is only rarely expressed in EBV-GC, EBV-encoded small RNA (EBER) is expressed in almost every EBV-GC cell, suggesting its importance for developing and maintaining this carcinoma. A recent study suggested that EBER activates insulin-like growth factor-1 in EBV-GCs. In addition, the hypermethylation-driven suppressor gene down-regulation, frequently observed in EBV-GC, appears to give a selective advantage for carcinoma cells. Prognostic significance of EBV involvement in gastric carcinoma is yet to be established, although there is a study reporting a favorable prognosis in EBV-GC.

In addition to p53 and beta-catenin expressions, we examined 59 EBV-GCs and 120 non-EBV-GCs for expressions of MUC1, MUC2, and MUC6, which have been reported as significant prognostic markers as well as phenotypic markers for gastric carcinoma, by immunostaining. All the phenotypic markers examined in this study were down-regulated in EBV-GC when compared to non-EBV-GCs, suggesting that most EBV-GCs may be classified phenotypically as null phenotype or gastric phenotype. Survival analysis revealed that lymph node metastasis and depth of invasion were significantly related to poor prognosis among non-EBV-GCs. On the other hand, intestinal type Lauren classification and tumor with MUC1-positive and MUC2-negative expressions, in addition to lymph node metastasis and depth of invasion, were significantly related to poor prognosis for EBV-GCs. Interestingly, nuclear and/or cytoplasmic expression of beta-catenin was associated with better prognosis of EBV-GC. Factors involved in the prognosis of EBV-GCs and non-EBV-GCs might be different from each other.

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IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF HPV-RELATED CERVICAL SQUAMOUS INTRAEPITHELIAL LESIONS: OLD AND NEW MARKERS

Evanthia Kostopoulou

Pathology Department, Medical School, University of Thessalia, Larissa, Greece

A large number of molecular studies in recent years has revealed multiple interactions between HPV oncoproteins and their cellular targets, which result in alterations of the cell cycle control, apoptosis and telomerase up-regulation. Increasing emphasis has been placed on the molecular analysis of HPV in cervical samples, with HPV typing, viral-load assessment and detection of HPV integration attracting the attention of most investigators. Although histopathologic evaluation remains the basic method for the diagnosis of HPV-related lesions and several studies have investigated the relationship of morphological findings to specific molecular events, the routine application of techniques assisting the diagnosis is limited.

Immunohistochemistry (IHC) has been employed, targeting antigens that are secondarily affected by the presence of HPV through several pathways. Since HPV oncoproteins induce alterations in the cell cycle, several associated markers have been investigated for their potential utility in assisting the histopathologic classification of squamous intraepithelial lesions and facilitating the distinction from non-HPV induced alterations. These included Ki67, PCNA, p16, cyclins and other molecules associated with regulation of the cell cycle.

In most laboratories immunostains for p16, a cyclin-dependent kinase inhibitor, and Ki-67 are the only routinely

used, cost effective surrogate markers for histopathologic diagnosis of HPV-related lesions. A review of published articles concerning p16 applications reveals several discrepancies and limitations. Positivity in squamous intraepithelial lesions varies from 14.3% to 100% in published studies and this variation could be attributed to: a) differences in the criteria for lesion diagnosis and for evaluation of immunohistochemical staining, b) differences in antibodies and techniques used, and c) geographic differences in the distribution of HPV types. Nevertheless, with increasing numbers of cases in different studies there appears a small group of high grade lesions that do not show any immunoreactivity. This observation diminishes the utility of p16 as a screening test.

Our laboratory has been recently involved in a project about cell cycle deregulation at G2M-transition checkpoint and beyond. Following some preliminary data we investigated the potential applications of cyclin B1 IHC in the diagnosis of cervical lesions and have recently shown that patterns of immunoreactivity for cyclin B1 correlate to lesion grade, while a pattern of cyclin B1 immunoreactivity in low-grade squamous intraepithelial lesions correlates strongly with the presence of HPV. Moreover, cyclin B1 immunoreactivity forecasted the detection of HPV by PCR in cases with dubious morphologic features, probably facilitating the recognition of "pre-koilocytes".

INCENP, the archetypal chromosomal passenger protein and a component of the chromosomal passenger complex, has been shown to be overexpressed in tumour cells and has recently been investigated by our group concerning its possible utility as surrogate marker in cervical biopsy diagnosis and its relationship with high-risk HPV types.

The potential applications of immunohistochemistry in the diagnosis of HPV-related cervical squamous intraepithelial lesions are not restricted to the above markers. Future studies could identify an ideal test or panel of markers which would reliably predict progression.

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SENSITIVE DETECTION OF OVEREXPRESSED TUMOR-ASSOCIATED STRUCTURES ON VARIOUS CANCER CELL TYPES

Beatrix Kotlan[1,2], Jozsef Timar[2] and Mepur H. Ravindranath[3], Mark C. Glassy[1,4]

[1]Integrated Medical Sciences Association Foundation, 10246 Parkdale Avenue, San Diego, CA 92126, USA;
[2]National Institute of Oncology, 1122 Rath Gy street 7-9, Budapest, Hungary;
[3]The Terasaki Laboratories, 11570 W. Olympic Blvd, Los Angeles, CA 90064, USA;
[4]University of California, MAE Department, La Jolla, San Diego; CA 92037, USA

Backgound and Objectives: One aim in cancer research is to reveal and detect novel tumor-associated membrane structures. Special acidic glycosphingolipids overexpressed on the malignant tissue have been shown to be important candidates for therapeutic issues. Monoclonal antibodies currently available are restricted in terms of specificities, and unique types of these antigens fail to be detected this way. The objective of this project was to overcome the difficulties concerning the detection of tumor-associated antigens with low immunogenicity. *Methods and Results:* Antibodies were obtained from different tissue origins and by different methods, that is hybridoma technology, EBV transformation and antibody engineering. Tumor cells of different hystological types were investigated in a standardised enzyme-linked immunosorbent assay (ELISA) and thin layer chromatoraphy (TLC) with dot blot technique. Indirect immunofluorescence (IF) assay with FACS analysis and modified chamber slide IF technique with confocal laser microscopy were performed to test for fine specificity and the degree of expression. The specific antibody-based immunological techniques were suitable to detect special characteristics of these acidic glycosphingolipids, highly associated with the cancerous tissue. *Conclusion:* The applied technique was suitable to define and bind to those tumor-associated cell surface structures that are difficult to reveal because of their weak immunogenicity. The results are promising, as tumor diagnostic and antibody-based therapy may be better formulated for the control of the disease.
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LIPOPLATIN PLUS GEMCITABINE *VERSUS* CISPLATIN PLUS GEMCITABINE AS FIRST-LINE TREATMENT AGAINST NSCLC: INTERIM ANALYSIS OF A PHASE III TRIAL

N. Mylonakis[1], A. Athanasiou[2], J. Angel[3], S. Lampaki[4], C. Kosmas[1], A. Rapti[5], N. Ziras[2], N. Politis[6], S. Kottaridis[7] and T. Boulikas[7]

[1]2nd Pathology Clinic, Metaxa Anticancer Hospital, Pireaus;
[2]1st Pathology Clinic, Metaxa Anticancer Hospital, Pireaus;
[3]Pulmonary Medicine Department, Theageneio Anticancer Hospital, Thessaloniki;
[4]Department of Medical Oncology, Aristotle University of Thessaloniki School of Medicine, Papageorgiou Hospital, Thessaloniki;
[5]8th Pulmonary Clinic, "Sotiria" Thoracic Hospital, Athens;
[6]Pulmonary Medicine Clinic, Agios Savvas Anticancer Hospital, Athens; [7]Regulon, Inc. Mountain View, California, USA and Athens, Greece

Background: Lipoplatin is a liposomal cisplatin, designed to reduce cisplatin toxicities without reducing efficacy. Its

nanoparticles evade immune surveillance, extravasate preferentially into tumors and metastases and target endothelial cells of tumor vasculature inducing cell apoptosis and antiangiogenesis. Human studies have shown a 40-200 fold higher concentration of platinum in tumor specimens after a single infusion compared to adjacent normal tissue. Lipoplatin has an orphan drug status for pancreatic cancer in the EU. This is the initial report of a randomized phase III trial. *Methods:* Eligibility criteria included inoperable/ metastatic NSCLC, no previous chemotherapy, WHO PS 0-1, adequate end-organ function. Patients received Lipoplatin 120 mg/m^2 D1,8,15 (Arm A) or cisplatin 100 mg/m^2 D1 (Arm B) with gemcitabine 1 g/m^2 D1,8, in 3-week cycles, with disease evaluation after 3 and 6 cycles. Primary endpoints were OS; secondary endpoints are ORR, DCR, PFS, and toxicity. Results: 88 patients treated, 47 with LipoGem and 41 with CisGem; 80 were evaluable. Response rates were (Arm A *vs*. Arm B): PR 37% *vs*. 28%, SD 34% *vs*. 31%, PD 29% *vs*. 41%. ORR was 37 *vs*. 28%, and DCR was 71% *vs*. 59%. PFS range is currently 0.7-16.8+ *vs*. 0.2-9.8+, and duration of response is 2.8-14.8+ *vs*. 2.1-10.4+ (months); final data are pending. The only grade IV adverse event in LipoGem was neutropenia in 2% of patients. Toxicities were (Arm A *vs*. Arm B): anemia I-III (94% *vs*. 100%), leucopenia III-IV (9% *vs*. 17%), neutropenia III-IV (11% *vs*. 29%), thrombocytopenia III-IV (9% *vs*. 22%), nephrotoxicity III (0% *vs*. 5%), nausea/vomiting III-IV (2% *vs*. 12%), neurotoxicity II-III (0% *vs*. 7%), asthenia III (4% *vs*. 17%), anorexia III (2% *vs*. 15%). Additionally, less antiemetics and G-CSF were administered in Arm A. *Conclusion:* Lipoplatin appears to have lower toxicity, mainly nephrotoxicity, as well as higher efficacy than cisplatin, when combined with gemcitabine in advanced NSCLC. Particularly relevant is that Lipoplatin is administered without pre- or post-hydration, on an outpatient basis.

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ASSOCIATION OF NUCLEOLI FRAGMENTATION WITH CISPLATIN RESISTANCE

G.K. Koumakis and J.G. Delinassios

International Institute of Anticancer Research, Kapandriti 19014, Greece

Background: Cisplatin has been one of the principal anticancer drugs for the last 3 decades and is widely used in the treatment of several cancers. However, it is known to be implicated in cases of development of drug resistance. It is well established that there are several mechanisms of drug resistance in cancer cells mostly involving increased production of specific proteins. The nucleolus represents a highly dynamic nuclear compartment, easily visible by light microscopy, directly involved in the fulfilment of the need for synthesis of large amounts of ribosomal RNA. Nucleolar morphology can be evaluated histologically using the silver staining method for nucleolar organizer regions (NORs). NORs are loops of ribosomal DNA within the nucleus that transcribe ribosomal RNA and are usually tightly aggregated within the nucleoli in interphase cells. Alterations in the number and configuration of NORs have been demonstrated in several human neoplasms and related to progression. In our previous study, NOR activity and morphology has been associated with the mechanism of the DHFR gene amplification during the development of resistance to Methotrexate in HeLa cells (Experientia, *39*: 1394, 1983, Med Oncol & Tumor Pharmacother, *2(1)*: 33, 1985, Experimental Cell Biology, *55*: 69, 1987). The aim of this work is to examine the NOR activity and morphology during development of resistance to Cisplatin *in vitro* in HeLa cells. *Methods:* Acquired resistance to Cisplatin was induced in HeLa cells by gradually increasing doses of Cisplatin starting from 0.05 μg/ml. HeLa clones resistant to Cisplatin 0.1 and 0.2 μg/ml were established over a period of 4 months. $AgNO_3$ staining was applied to resistant HeLa cell lines and their sensitive counterparts. Morphometric analysis of 100 HeLa cells for each Cisplatin resistance level was applied for 2-D measurement (pixels^2) of stained regions using Axiovision's LE morphometry application. The organization patterns of NORs were investigated numerically (mean NOR count) and morphologically (nuclear size, NORs size, configuration). *Results:* Non-resistant HeLa cells exhibited usually 1-2 large and round and 1-3 small nucleoli (Figure 1AB). HeLa cells resistant to Cisplatin 0.1 μg/ml exhibited a higher number of nucleoli with irregular shapes (Figure 1CD). HeLa cells resistant to Cisplatin 0.2 μg/ml exhibited remarkably fragmented nucleoli. One large irregularly shaped and numerous small nucleoli were present in 100 % of the cells (Figure 1EF).

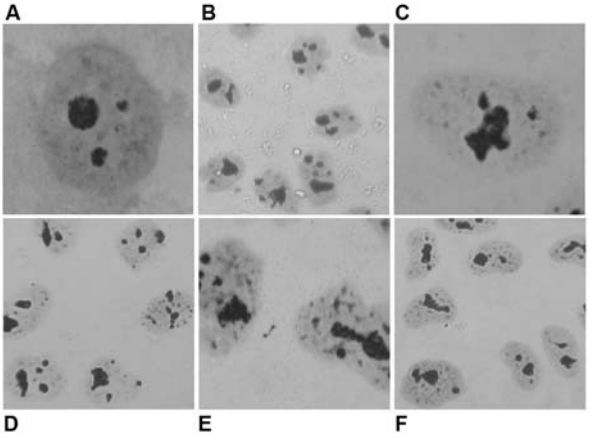

Figure 1. *NORs in HeLa cells using AgNOR staining. (A,B) Non-resistant cells [0 μg/ml Cisplatin], (C,D) Resistant cells [0.1 μg/ml Cisplatin], (E,F) Resistant cells [0.2 μg/ml Cisplatin].*

Measurement of the total 2-D NOR surface in the non-resistant and the two resistant HeLa cell lines did not show a significant difference. However, if the number of individual NORs in each nucleus were counted separately and independently of size, a very significant difference was noted between the non-resistant and the Cisplatin 0.2 μg/ml resistant HeLa cells (Table).

Table. *Morphometric analysis measurements of HeLa resistant and non-resistant cells.*

Cisplatin (μg/ml)	0	0.1	0.2
Mean AgNOR Count	3.82	4.19	12.36
Morphology/Shape	Regular	Irregular	Irregular
Mean Size			
Total Cell surface ($pixels^2$)	598500	594000	581500
Mean Size			
Total NORs surface ($pixels^2$)	79200	78900	79100
TOTAL/NOR	7.556	7.528	7.351
[TOTAL-NOR]/NOR	6.556	6.528	6.351

The Cisplatin 0.1 μg/ml resistant cell nuclei showed a total number of NORs rather close to non-resistant cell nuclei. However, the morphology of the large nucleoli was irregular and gave the impression of an aggregation of many smaller nucleoli resembling a "bunch of grapes". *Discussion and Conclusion:* The present results showed that the development of resistance to Cisplatin is associated with obvious changes in the morphology of NORs in interphase nuclei of HeLa cells. The total surface of the nucleoli in the Cisplatin 0.2 μg/ml resistant HeLa cells is obviously larger in comparison to the non-resistant cells. Therefore, we may infer that the increased surface of the nucleoli, due to their extensive fragmentation, is associated with the mechanisms of resistance to Cisplatin. Whether, or not this feature is accompanied with a higher production of rRNA should be investigated.

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HELICOBACTER PYLORI INVOLVEMENT IN UPPER AND LOWER GI TRACT ONCOGENESIS IN GREECE

Jannis Kountouras

Department of Medicine, Second Medical Clinic, Aristotle University of Thessaloniki, Ippokration Hospital, Thessaloniki, Greece

Gastric adenocarcinoma not located in the cardia still remains second only to lung cancer as leading cause of cancer-related mortality worldwide, although adenocarcinoma of the cardia and gastroesophageal junction has been rising rapidly over the past two decades. Gastric malignancy can be subdivided into diffuse and intestinal pathological entities that have different epidemiological and prognostic features. Various genetic and environmental factors lead to either abnormal genes overexpression or inappropriate expression of normal genes, whose products confer the malignant phenotype. *Helicobacter pylori* (*H. pylori*) infection appears to be involved in gastric carcinogenesis through various molecular mechanisms, including repopulation of the stomach with bone marrow-derived stem cells (BMDSC) that may facilitate gastric cancer progression, thereby necessitating eradication of this bacterium. In addition, *H. pylori* might be involved in the gastroesophageal reflux disease – Barrett's esophagus (BE) – esophageal adenocarcinoma (EA) sequence and its eradication might inhibit the progress of this sequence. Although EA is now the most common esophageal malignancy in Western countries whose incidence is increasing faster than any other cancer, mortality from esophageal cancer in Greece is among the lowest in the world and no clear-cut answer has emerged as to why the incidence of EA is so low in Greece. Our findings indicate the possible existence of a balance between cell proliferation (indicated by Ki-67 increased expression) and apoptosis (indicated by Bax protein overexpression) in BE patients, thereby providing an equilibrium between cell apoptosis and cell proliferation, and this may partly explain the low EA incidence in Greece. Moreover, some reports have indicated an increased risk of colorectal cancer in patients with BE or EA. A possible association of both diseases might be explained by genetic predisposition or common environmental risk factors possibly including *H. pylori* infection. Our findings established the presence of *H. pylori* in cancer tissues of the majority of colorectal cancer patients, and *H. pylori* infection might also recruit BMDSC that may ultimately facilitate colon cancer progression. Contrary to the data of other countries, the incidence of cancer in Greek patients with inflammatory bowel disease (IBD) also appears to be low. In this respect, our preliminary results also suggest the possible existence of a balance between cell pro-apoptotic and anti-apoptotic mechanisms in ulcerative colitis, indicated by Bax and Bcl-2 overexpression in 50% of the patients, and a great propensity of pro-apoptotic mechanisms in Crohn's disease, indicated by Bax and Bcl-2 overexpression in 62% and 25% of the patients respectively, thereby reflecting a coherent apoptotic process that might explain, at least in part, the low incidence of cancer development in Greek IBD patients.

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INFLUENCE OF TWO *LEGUMINOSAE* PLANT EXTRACTS ON GROWTH AND ANTIOXIDANT DEFENSE SYSTEM OF Hep2 CANCER CELL LINE

C. Spanou and D. Kouretas

University of Thessaly, Department of Biochemistry and Biotechnology, 41221, Larissa, Greece

Legumes are considered to be a very good source of phytochemical compounds that may act as chemopreventive agents especially by their antioxidant properties (1). In a previous report we examined the antiradical and protective properties against free radical-induced DNA damage of extracts derived from various Greek *Leguminosae* family plants (2). From the results obtained two extracts showing potent antioxidant properties were chosen for further study. Aqueous extracts of aerial parts of *Lathyrus laxiflorus* and *Phaseolus vulgaris* plants were initially examined for their cytotoxicity properties on Hep2 cancer cell line. Their cytotoxicity was assessed at concentrations 100, 400, 800 μg/ml after 24h incubation with the extracts. *Lathyrus laxiflorus* plant extract exhibited 57% and 74% inhibition of cell growth at concentrations 400 and 800 μg/ml respectively, whereas *Phaseolus vulgaris* extract had no effect on cell growth in none of the tested concentrations. IC_{50} values for *Lathyrus laxiflorus* were 390 μg/ml and 4.9 μg/ml against $OH^{\bullet}$ and $ROO^{\bullet}$ radicals respectively, whereas for *Phaseolus vulgaris* against the same radicals were >1600 μg/ml and 17 μg/ml (2).

Non cytotoxic concentrations, 100 μg/ml of *Lathyrus laxiflorus* and 800 μg/ml of *Phaseolus vulgaris* extract, were used for 2, 12, 24 hours incubation of the cells. The influence of the extracts on the antioxidant defense system of the cells was assessed by measuring the total antioxidant capacity of the cells (TAC) and the amounts of catalase (CAT), glutathione (GSH), oxidized form of glutathione (GSSG) and thiobarbituric reactive substances (TBARS) in all times of incubation of the cells. From the results obtained it seems that only *Lathyrus laxiflorus* extract induces oxidative stress in the cells by reducing TAC, and CAT and inducing TBARS especially in 2 and 12h of incubation. *Phaseolus vulgaris* extract reduced only TAC at 2h of incubation indicating also a mild induction of oxidative stress. These results imply that potent antioxidant extracts, beyond a critical concentration, may induce oxidative stress and cytotoxicity in cells which shows that antioxidant activity of various chemicals should be considered with caution.

1 Rochfort S *et al*: J Agric Food Chem *55*: 7981-7994, 2007.
2 Spanou C *et al*: Anticancer Res *27*: 3403-3410, 2007.

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DESIGNING ANTIBODY-MAYTANSINOID CONJUGATES FOR ANTICANCER THERAPY

Yelena Kovtun

Senior Scientist, ImmunoGen, USA

Conjugation of cytotoxic compounds to antibodies allows targeted delivery of the cell-killing agents to tumor sites. Numerous conjugates of antibodies with maytansinoids, derivatives of a microtubule-binding compound, are in development and several antibody-maytansinoid conjugates are in clinical trials. Modifications of the linker that connects the maytansinoid to the antibody impact the conjugate metabolism, bystander killing, pharmacokinetic parameters and intracellular retention of cytotoxic metabolites. The specific design of conjugates for the treatment of multidrug-resistant cancer and tumors that express the target antigen in a heterogeneous manner is discussed.

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PRACTICAL USE OF TELEPATHOLOGICAL CONSULTATION TO IMPROVE CANCER INCIDENCE STATISTICS

András Huszár, Antal Kricskovics and Ágnes Frick

University of Pécs Faculty of Medicine Department of Forensic Medicine, 7624 Pécs, Szigeti út 12, Hungary

It is a widely observed fact that the number of clinical autopsies around the world are decreasing, therefore their relevance in providing accurate statistics for the incidence of cancers is also getting weaker. However, the number of medico-legal autopsies in our Institute are growing. This tendency has been noted by several other universities. Our goal is to help improve the accuracy of these statistics by documenting all accidentally found tumors during a medico-legal autopsy, because these were not included in the statistics numbers up to now. To gain useful data from these specimens, the histological slides have to be diagnosed by a pathologist, however, there is no one with such a qualification in our institute. The most promising solution for our endeavour in telepathological consultation is the use of digital microscopy. It permits the digital modelling of routine histological slides and it also allows measurements by using analysis or stereology software packages. The digital slide is nowadays a fast and accessible item due to fully established broadband internet and local area networks (LAN). The MIRAX Desk (Carl Zeiss, Germany) scanner we use turned out to be an effective tool due to its high quality images and fast scanning process. By using the digital slide database PathoNet (www.pathonet.org) and the MIRAX Viewer

software we were able to set up a fast telepathological consultation process, which can connect us with any partners around the globe with network access, hereby providing an exact diagnosis of our specimen.

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TARGETING CELL SURVIVAL AND APOPTOTIC SIGNALING PATHWAYS FOR PROSTATE CANCER MANAGEMENT

Rita Ghosh, Ganapathy Manonmani, Muralimanoharan Sribalasubashini, Heather Graham, Paul Rivas, Xie Jianping, Craig Robson and Addanki P. Kumar

Department of Urology University of Texas Health Science Center at San Antonio, TX, USA

Prostate cancer (PCA) is the second leading cause of cancer-related deaths in men in western society. While African American men have the highest incidence of prostate cancer in the world Asian men native to their countries who consume low fat and high fiber diet have the lowest risk. Migration of Asians to Western countries puts them at high risk for prostate cancer. Epidemiological studies also suggest that a reduced risk of cancer is associated with the consumption of phytochemical-rich diet that includes fruits and vegetables. Strategies to delay clinically significant prostate cancer will have a tremendous impact in reducing the overall incidence of prostate cancer as well as increasing quality of life for elderly men. The long latency involved in the development of clinically significant prostate cancer provides plethora of opportunities for its management especially using prevention approaches. In addition cancer arises due to deregulation of multiple signaling pathways, thus targeting multiple signaling pathways using a combination of agents or complex botanicals have an added advantage in providing synergistic or additive effects. Studies conducted in our laboratory show that Nexrutine^R (bark extract from *Phellodendron amurense*) inhibits proliferation of prostate cancer cells and prostate tumor development in the transgenic adenocarcinoma of mouse prostate (TRAMP) model through modulation of Akt signaling pathway. To further define the mechanism of action of Nexrutine^R and to identify the active component associated with its biological activity using activity guided fractionation we have identified butanol fraction as the active component of Nexrutine^R. Butanol fraction recapitulated the activities of Nexrutine^R in (i) inhibiting proliferation; (ii) inducing apoptosis; and (iii) modulating transcriptional activity of NFκB in prostate cancer cells. Our data also indicates that both Nexrutine^R and butanol fraction modulates NFκB transcriptional activity by inhibiting IκBα phosphorylation. Expression of p65 and phosphorylated IκBα are high in tumors from TRAMP mice. In contrast dietary administration of Nexrutine^R reduced expression of p65 and phosphorylated IκBα in prostate from TRAMP mice that correlated with inhibition of tumor development. In addition using ultra-performance liquid chromatography we have identified berberine or closely related compound may be responsible for the observed biological activities in the butanol fraction that may induce apoptosis in prostate cancer cells by targeting critical cell survival signaling pathways both *in vitro* and *in vivo*.

Studies from our laboratory also identified potential role for Sp1-FLIP (FADD-Like interleukin-1β-converting enzyme (FLICE) inhibitory protein) signaling for modulation of apoptosis in prostate cancer cells. Data to demonstrate role for FLIP signaling or Akt/CREB signaling in prostate cancer prevention efforts will be discussed. Supported in part by NIH R21 CA 98744; ACS RSG-04-169-01 (APK).

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A NOVEL INVESTIGATION OF PAX3 FUNCTION IN EMBRYONIC CELL LINES UNDERGOING DIFFERENT DIFFERENTIATION PATHWAYS AND THE CORRESPONDING TUMOURS

Patricia Kumar[1], Qiuyu Wang[1], Hongmei Li[1,2], Wen-hui Fang[1,2], Mark Slevin[1] and Shant Kumar[2]

[1]Department of Biology, Chemistry and Health Science, Manchester Metropolitan University, Chester St, Manchester, M15GD;
[2]Division of Laboratory and Regenerative Medicine, Faculty of Medicine and Human Sciences, Manchester University, Oxford Road, Manchester, M13 9PT, UK

PAX3 is a transcription factor expressed for only a few days during embryonic development of the neural crest and dorsal dermomyotome, but re-expressed in tumours of cells derived from these embryonic populations. We have up-regulated PAX3 expression by transfection into murine melanocytes, myocytes and embryonic stem cells for comparison with PAX3 down-regulation using siRNA in the corresponding tumours: melanoma, rhabdomyosarcoma and neuroblastoma. We have studied three of the seven PAX3 isoforms which we isolated previously- namely PAX3 c, e and g.

The effects of PAX3c, e or g, singly, *versus* an empty vector control were determined in transfected cells using assays for cell proliferation, migration, apoptosis and adhesion. Affymetrix microarrays identified genes up- or down-regulated by each of the three isoforms. The relevance of these genes of interest was confirmed using RT-PCR and western blotting.

Microarray results are being compared between 1) an embryonic cell line and its corresponding tumour type 2)

melanogenesis, myogenesis and embryonic stem cell differentiation into sympathetic neurones. Genes up-regulated in embryonic cells while being down-regulated in the corresponding tumours are likely to be important downstream targets of PAX3. Genes regulated in the same way by three different isoforms might also be important. PAX3 might use different target genes in cells following different differentiation pathways.

It is hoped that this study will illuminate the role of PAX3 in cancer and identify important molecular targets to be used in therapy.

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TELOMERASE ACTIVITY AS A DIAGNOSTIC AND PROGNOSTIC FACTOR IN HEAD AND NECK CANCER

Zuzana Kunická[1,2], Igor Mucha[3] and Jiří Fajkus[1,2]

[1]Department of Functional Genomics and Proteomics, Institute of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, CZ-62500 Brno; [2]Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, CZ-61265 Brno; [3]St. Ann's University Hospital, Pekarska 53, CZ 656 91 Brno, Czech Republic

Activation of telomerase is tightly associated with many types of cancer, including head and neck cancer. We examine the use of telomerase activity as a diagnostic and prognostic marker of head and neck cancer development in comparison with standard histological analysis.

Telomerase activity was determined using quantitative Dual-Colour Real-Time TRAP. In each of 58 patients, a sample of tumour tissue and adjacent mucosa was collected. As a control, we collected samples of normal muscle tissues.

Telomerase activity was observed in 88% of tumour tissues and 34% of tumour-adjacent mucosa samples. No telomerase activity was detected in normal muscle tissues. Telomerase activity correlated with tumour grade, showing an average of 4.6 telomerase units (T.U.) in well-differentiated, 8.3 T.U. in moderately-differentiated and 20 T.U. in poorly differentiated tumours. Relapse occurred in 13 patients and no telomerase activity was detected in 3 recurrent tumours.

Telomerase activity may be used as an objective parameter inversely related to tumour differentiation. Prognosis in telomerase-negative tumours is worse than that of the telomerase-positive group due to a higher frequency of recurrences.

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BAICALEIN-INDUCED APOPTOSIS AND INHIBITED METASTASIS OF HUMAN HEPATOMA J5 CELLS

Hsiu-Maan Kuo[1], Hung-Jie Tsai[1], Ya-Ling Lin[1] and Jing-Gung Chung[2]

Departments of [1]Parasitology and [2]Biological Science and Technology, China Medical University, Taichung, Taiwan, ROC

Although baicalein has been demonstrated to induce apoptosis in many human cancer cell lines, the exact molecular mechanism of apoptosis induced by baicalein in human liver cancer J5 cells is unknown. The purpose of this study was to investigate whether or not baicalein affects the cell cycle and induces apoptosis in J5 cells by flow cytometry. DAPI staining and Comet assay were used to examine the DNA damage. Confocal laser microscope was used to examine the translocation of AIF and Endo G from mitochondria to nuclei. Western blotting was used to examine the associated proteins in cell cycle and apoptosis. The results indicated that baicalein induced G_2/M-phase arrest through the phosphorylation of cdc25c and cdc2, and inhibited cdc2-cycin B1 complex. Baicalein promoted the productions of reactive oxygen species (ROS) and Ca^{2+} and reduced the change in the levels of mitochondrial membrane potential in J5 cells. Baicalein-induced apoptosis also caused AIF and Endo G release from mitochondria and promoted the activations of caspase-9 and caspase-3. Baicalein caused oxidative stress and Ca^{2+} release from ER and promoted GADD153 and GRP78 expression. ROS inhibitor (NAC) abrogated baicalein-induced effects on ROS and apoptosis baicalein led to decrease the production ROS and percentage of apoptosis. Matrix metalloproteinase (MMPs), one of the families of enzymes that degrade the extracellular matrix (ECM), are considered to play an important role of tumor invasion and spread. We have demonstrated that baicalein inhibits the invasion of J5 through the inhibition of MMP-9.

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DEFINING THE ROLE OF THE TYPE-1 INSULIN-LIKE GROWTH FACTOR RECEPTOR (IGF-IR) SIGNALING IN CHILDHOOD RHABDOMYOSARCOMA CELLS *IN VITRO* UNDER HYPOXIC GROWTH CONDITIONS

Raushan T. Kurmasheva and Peter J. Houghton

St. Jude Children's Research Hospital, Memphis TN 38105, USA

Background: IGF-IR signaling in rhabdomyosarcoma (RMS) cell lines is thought to be dysregulated by overexpression of the IGF-II ligand. However, functional linkage between IGF-

IR signaling and activation of a critical node in signaling, Akt, has not been defined. Further, the role of IGF-1R signaling in secretion of VEGF or IGF-2 is unknown. *Methods:* RMS cell lines were grown *in vitro* under normoxic (21% O_2) or hypoxic (1% O_2) conditions. Cells were treated for 24 h with CP751871 (1 μg/ml), an antibody targeting IGF-IR. IGF-IR, Akt, GSK-3β, S6 and their phosphorylated derivatives were determined by Western blot. Levels of VEGF and IGF-2 were determined by ELISA. *Results:* Under serum-free conditions CP751871 treatment completely blocked IGF-I stimulation of Akt phosphorylation in all cell lines. Under normoxic conditions in serum-containing media, treatment caused down-regulation of IGF-IR in 3 of 4 RMS lines but only slightly reduced p-Akt levels. Under hypoxic conditions phosphorylation of IGF-IR was significantly increased and CP751871 treatment markedly inhibited p-Akt in 3 out of 4 RMS lines. These results indicate the predominant pathway activating Akt under hypoxia is up-regulation of IGF-IR signaling. Of note, while cellular proliferation slowed under hypoxia, signaling through mTORC1 (the mTOR-raptor complex) was not attenuated, as S6 protein remained hyperphosphorylated. Under these conditions there was no increase in apoptosis in any cell line with or without CP751871. Furthermore, CP751871 had relatively modest effect in reducing hypoxia-driven increases in VEGF secretion, and no significant effect on hypoxia-driven increases in IGF-2 secretion. *Conclusion:* Our results suggest that CP751871 treatment potently inhibits IGF-IR signaling *in vitro*. Under normoxia, inhibition of IGF-IR has relatively small effect on p-Akt levels, suggesting alternate receptor-mediated pathways signal to Akt. In contrast, under hypoxic conditions, secreted IGF-2 levels increase and activate IGF-IR, leading to activation of Akt. Under these conditions, there is tighter linkage between IGF-IR signaling and Akt activation, suggesting a switch to IGF-IR dependence. Current studies are focused on defining whether CP751871 equally inhibits IGF-1 and IGF-2 ligand activation of IGF-IR under hypoxia.

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THE DOUBLE HAZARD OF BLEEDING AND THROMBOSIS IN CANCER PATIENTS

Hau C. Kwaan and Marjorie Barnett

Hematology-Oncology, Northwestern University Feinberg School of Medicine, Chicago, IL. 60611, USA

Since Trousseau first pointed out the association between thrombosis and cancer, both thrombosis and bleeding in cancer has been extensively studied. Major advances in basic and clinical investigations have enhanced our understanding of the pathogenesis of these complications. Recent epidemiological studies have also clarified the incidence of thrombo-embolic complications in different types of cancer, with notably higher incidence in malignant hematological disorders. The present day concept of thrombogenesis in cancer remains to be based on Virchow's original triad of aberrant blood flow, loss of vascular integrity and altered blood components, but with added knowledge of the influence of cytokines, growth factors and prothrombotic adverse effects of therapeutic agents and vascular access catheters. Additional prothrombotic risk factors include hereditary thrombophilia, infection, endothelial cell activation, antiphospholipid syndrome and acquired activated protein C resistance. While most cancer patients experience bleeding at some time during the course of their illness, there are special situations that increase bleeding diathesis. These include thrombocytopenia, endothelial injury, disseminated intravascular coagulation, excessive fibrinolysis, acquired hemophilia and adverse effects of drugs. Recognition of these factors will help with the adoption of appropriate prophylactic and therapeutic measures.

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RATE-LIMITING MICROENVIRONMENTS IN TUMOR BIOLOGY

Silvia Doratiotto, Fabio Marongiu, Paolo Pani, Sergio Laconi and Ezio Laconi

Department of Biomedical Sciences and Technology, University of Cagliari, Cagliari, Italy

Cancer is often the result of a stepwise, chronic disease process encompassing an extensive segment of the lifespan of any species. A common pathway in the natural history of the disease is the appearance of focal proliferative lesions that are known to act as precursors for cancer development. It is becoming increasingly apparent that the emergence of such lesions is not a cell-autonomous phenomenon, but is heavily dependent on microenvironmental cues derived from the surrounding tissue. Specific alterations in the tissue microenvironment that can foster the selective growth of focal lesions will be discussed.

Furthermore, we argue that a fundamental property of focal lesions as it relates to their precancerous nature lies in their altered growth pattern as compared to the tissue where they reside. The resulting altered tissue architecture translates into the emergence of a unique tumor microenvironment inside these lesions, associated with altered blood vessels and/or blood supply which in turn can trigger biochemical and metabolic changes fueling tumor progression.

From this perspective, the slow build-up of alterations in the tissue and tumor microenvironments represent rate-limiting

steps in cancer development. A deeper understanding of the role(s) of these changes in the pathogenesis of neoplasia is essential to design more effective strategies for the management of this disease.

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LOW-DOSE PHYTOTHERAPEUTIC COMPLEXES TO CONTROL CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

Alberto Laffranchi

Fondazione IRCCS Istituto Nazionale Tumori di Milano
Via Venezian 1, I-20133 Milano, Italy

For many chemotherapy regimens, neurotoxicity is the most important non-hematological dose-limiting toxicity and there are no guidelines for the treatment of chemotherapy-induced peripheral neuropathy.

We tested the efficacy of a low-dose phytotherapeutic complex (Arnica compositum, Rhododendroneel S, Ranunculus homaccord and Colocynthis homaccord), in patients with peripheral pain following chemotherapy, prescribing 10 drops of each alcoholic preparation in the evening. We first tested the treatment in 7 patients with post-taxane neuropathic pain in the hands, present for at least 3 months, refractory to gabapentin. In all cases, we observed a clinically significant reduction in hand pain about 3 months after initiating the treatment. We next successfully treated 5 breast cancer patients, with post-taxane neuropathic pain which had been present for about a month and 2 lung cancer patients with neuropathy of the hands and feet who experienced pain reduction within 20 days of initiating treatment. We subsequently treated two further patients one with lung cancer and the other with colon cancer. The former had had neuropathy of the hands and feet for over a year, the second had a similar complaint for two years following treatment with oxaliplatin and 5-fluorouracil which worsened at night and disturbed sleep. The patient with lung cancer stopped taking the treatment after 20 days for lack of benefit. In the patient treated for colon cancer, the symptoms in the hands resolved within 2 months but the problems in the feet persisted. In this patient we started weekly treatment with an injectable phytotherapeutic preparation containing *Acer negundo, Condurango, Fraxinus americana, Gallae, Haematoxylon campechianum, Lycopodium, Prunus padus, Raphanus, Scrofularia nodosa, Thuia, Ulmus campestris,* and *Viscuma album* (P73 Juv 110). The foot paresthesia improved after the first injection and further improved (no longer interrupted sleep) after the second injection. A third injection is planned and after that the oral treatment will continue.

The encouraging results with these low cost phytotherapeutic preparations indicate that controlled studies should be performed to validate their efficacy.

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RNA TECHNOLOGIES FOR REVERSAL OF ABC TRANSPORTER-MEDIATED MULTIDRUG-RESISTANCE IN CANCER

Hermann Lage

Charité Campus Mitte, Institute of Pathology, Charitéplatz 1, D-10117 Berlin, Germany

ABC transporters can mediate the multidrug-resistance (MDR) phenotype of human cancer cells. Thus, disruption of ABC transporter-mediated drug extrusion results in a re-sensitization of tumor cells to drug treatment. Low molecular weight compounds may circumvent MDR by inhibiting the efflux pump activities of the transporters. However, the innate side-effects of these compounds must be carefully considered. Thus, experimental gene therapeutic approaches using RNA technologies have been applied for inhibition of different ABC transporters. These techniques include antisense oligonucleotides, ribozymes and chemically synthesized small interfering RNAs (siRNAs) or expression cassette encoded short hairpin RNAs (shRNAs) mediating the RNA interference (RNAi) phenomenon. In order to reverse different types of MDR, different ribozymes and siRNAs were designed to inhibit the expression of the ABC transporters MDR1/P-gp, MRP2, and BCRP. These RNA constructs were used to treat different ABC transporter expressing cancer cell lines derived from various tissues. All RNA constructs decreased the level of specific ABC transporter mRNA and protein expression dramatically, and reduced the cellular resistance to drug treatment by enhancing the cellular drug accumulation *in vitro*. Since all of the anti-ABC transporter RNA constructs showed gene-silencing activity, plasmid expression vectors were designed for stable expression of shRNA molecules. Furthermore, expression vectors were designed that encode a multitarget multiribozyme (MTMR) or a multitarget-multi-siRNA/ribozyme (MTMsiR) simultaneously directed against three different ABC transporters, MDR1/P-gp, MRP2, and BCRP. Transfection of these RNA constructs into different MDR cell lines fully inhibited ABC transporter expression at the mRNA and protein level and completely reversed the drug-resistant phenotypes. For *in vivo* application, shRNA encoding DNA was designed to reverse ABC transporter-mediated MDR phenotype. An adenovirus- and an *Escherichia coli*-basing strategy, as well as a nonviral jet-injection technology were developed for *in vivo* delivery of shRNA-vector constructs.

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RADIATION FROM SUN AND SUN-BEDS: CARCINOGENIC AND ANTICARCINOGENIC EFFECTS

Johan Moan, Z. Lagunova and A.C. Porojnicu

Norwegian Radium Hospital, Institute for Cancer Research, Department of Radiation Biology, Montebello, 0310 Oslo, Norway

All life, certainly including human life, has been developed under solar radiation. Humans and their ancestors have spent most of their time under the strong fluence rates of the Equatorial sun, and are adapted to those. In fact, the action of solar radiation is so important for human health that the skin color changed from black to white when humans migrated from their Equatorial home place to higher latitudes. In spite of this evolutionary fact, the medical literature tells that our attitude towards solar radiation has oscillated like a pendulum: from fear and avoidance to love and worship and back to fear again. The reason for this is certainly that solar radiation has both positive and negative effects; all depends on exposures and exposure patterns. With our current knowledge of action spectra and dose-effect relationships for positive and negative health effects, we should be able to quench the oscillations and settle at an intelligent behavior of optimal equilibrium. Skin carcinogenesis is certainly the major negative effect of sun exposure, while vitamin D formation is linked to a number of positive effects, one of which is anticarcinogenesis. Action spectra for these effects will be reviewed: While those for vitamin D formation, for pigmentation, for erythema and for non-melanoma generation are quite similar and located in the UVB region, that for melanoma generation has a significant contribution in the UVA region. This has important health consequences: UVA has a much smaller latitude gradient than UVB and decays less with time before and after noon. In view of this it is possible to find a time and an exposure pattern that is optimal for vitamin D generation at a minimal risk of melanoma.

From our epidemiological studies we conclude that for European countries a moderate increase in non-erythemal sun exposure for those with indoor work would be associated with much larger positive than negative health effects. For instance, in Norway an increased exposure, leading to 200 more melanoma deaths per year (a doubling) if non-optimally obtained, would probably lead to more than 2000 fewer internal cancer deaths.

If the early humans had known sun-beds, all of us would probably still have been black. Our fear of "artificial devices" like sun-beds, should be reevaluated in view of the fact that at a given wavelength it is impossible to distinguish photons from a sun-bed from photons from the sun. It is possible to construct sun-beds with spectra that are much more "healthy" than those of the sun. Such spectra would be stable and would not vary with time and localization like the spectra of the sun.

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LYSOSOMES AND PROTEOLYTIC SIGNALLING IN TUMOUR PROGRESSION

Tamara T. Lah, Maria-Beatriz Durán Alonso, Boris Gole, Saša Kenig, Anja Pucer and Irena Zajc

Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia

Altered lysosomal functions in tumour progression have not yet been completely revealed. These vesicles contain various hydrolytic enzymes, including cathepsins, which are involved in several cellular processes, both intracellularly as well as extracelluly after cathepsin secretion. Therefore, the interactions of tumour cells with their microenvironment, for example in angiogenesis, are also mediated *via* cathepsins in addition to processes such as tumour cell invasion, apoptosis and drug resistance. These processes are associated with partial or extensive degradation of protein substrates, trafficking and recycling of relevant biological molecules, such as receptors and growth factors, and post-translational modifications of secretory proteins. A broad pH optimum and selective specific activity of these enzymes, being tuned by at least three cystatins (stefins A and B and cystatin C), allow them to participate in biochemical mechanisms underlying tumour progression. Recent experiments using various methods for knocking out and silencing cathepsin genes in normal and tumour cells have clearly revealed differential expression and novel functions of these enzymes. They also affect gene regulation in the tumour and in the surrounding cells, clearly depending on the cell type. A review of recent experimental and clinical findings in glioma will be presented. It is concluded that, at least in brain tumours, cathepsin B supports tumour cell invasion and angiogenesis while cathepsin L is more associated with apoptosis and drug resistance, whereas the role of cathepsin S is at present less clear.

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KEY ISSUE IN RESPECT TO STABILITY OF CYTOKERATIN18 IN HEPATOCELLUAR CARCINOMA

Chiung-Chi Cheng[1], Yi-Hsiang Liu[2,3], Chin-Chin Ho[4] and Yih-Shyong Lai[5]

[1]Institute of Medicine of Chung Shan Medical University, Taichung, Taiwan;
[2]Department of pathology, Jen Ai Hospital, Taichung, Taiwan;

[3]Department of Pathology, Tzu Chi Hospital and University, Hualien, Taiwan;
[4]Department of Nursing, Central Taiwan University of Science and Technology, Taichung, Taiwan;
[5]Department of Pathology, Hospital and Medical College of Chung Shan Medical University, ROC

Intermediate filaments are important in building cellular architecture. Previously we found cytokeratin18 was modulated in human hepatocellular carcinoma. Plectin is a cross-linking protein that organizes the cytoskeleton into a stable meshwork, which can maintain the uniform size and shape of hepatocytes. Because the cells of hepatocellular carcinoma were morphologically different from the hepatocytes, we speculated that expression of plectin and organization of intermediate filament might play roles in the pleomorphism of hepatocellular carcinoma cells. We studied the plectin expression of hepatocellular carcinoma and liver tissues by immunohistochemistry and immunoblot. The results revealed that plectin was deficient and cytokeratin18 was modulated in hepatocellular carcinoma. Furthermore, the knockdown the plectin mRNA in Chang cells, revealed that plectin was deficient and the organization of cytokeratin18 was altered. Conclusively, this study offers a hypothesis that plectin deficiently might play an important role in the tumorigenesis of hepatocellular carcinoma.

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STROMAL FGF-2 PARTICIPATES IN HORMONE INDEPENDENT TUMOR GROWTH

Juan Pablo Cerliani[1], Sebastián Giulianelli[1], Victoria T. Fabris[1], Virginia Novaro[1], Adrián Góngora[1], Alberto Baldi[1], Alfredo Molinolo[2], Claudia Lanari[1] and Caroline A. Lamb[1]

[1]Instituto de Biología y Medicina Experimental, Vuelta de Obligado 2490, Buenos Aires, Argentina;
[2]National Institutes of Health/NIDCR, Oral and Pharyngeal Cancer Branch, Building 30 Room 211, 30 Convent Dr MSC 4340, Bethesda MD 20892-4340, USA

We have developed an experimental model of breast cancer in BALB/c female mice in which metastatic ductal mammary carcinomas transit through different stages of hormone dependence. Hormone-dependent (HD) tumors need the exogenous administration of progestins to grow while hormone-independent (HI) carcinomas grow *in vivo* without exogenous progestin supply, although they retain high levels of estrogen and progesterone receptors (PR). *In vitro*, however, there are no differences in hormone responsiveness between both tumor types, suggesting the involvement of host factors regulating *in vivo* tumor growth.

The mechanisms by which mammary carcinomas acquire hormone independence are still unknown. The aim of this study was to evaluate the role of carcinoma-associated fibroblasts (CAF) in the acquisition of hormone independence. We demonstrated that CAF from HI tumors (CAF-HI) growing *in vitro* express higher levels of FGF-2 than HD counterparts (CAF-HD). FGF-2 activated the PR in the tumor cells, thus increasing cell proliferation. CAF-HI induced a higher proliferative rate in the tumor cells and in PR activation than did CAF-HD. The blockage of FGF-2 in the co-cultures, and the genetic or pharmacological inhibition of FGFR-2 inhibited PR activation and tumor cell proliferation. *In vivo*, an FGFR inhibitor reduced HI tumor growth, and exogenous administration of FGF-2 to HD tumors promoted growth. T47D human breast cancer cells were also stimulated by progestins, FGF-2 or CAF-HI, and this stimulation was abrogated by antiprogestins, suggesting that the murine C4-HI cells respond as the human T47D cells.

In summary, this is the first study reporting differences between CAF from HD and HI tumors suggesting that CAF-HI actively participate in driving HI tumor growth.

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OBESITY PROMOTES GASTROINTESTINAL CANCER IN *Apc* MUTANT MICE

Claudia Gravaghi, Jin Bo, Krista MD LaPerle, Fred Quimby, Raju Kucherlapati, Martin Lipkin, Winfried Edelmann and Sergio Lamprecht

Strang Cancer Research Laboratory, Division of Gastroenterology and Hepatology, Department of Medicine, Weill Medical College of Cornell University, New York, NY, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY, Laboratory of Comparative Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, Laboratory Animal Research Center, The Rockefeller University, New York, NY, Department of Genetics, Harvard-Partners Center for Genetics and Genomics, Boston, MA, USA

Epidemiological studies indicate a link between obesity and colon cancer.

The main objective of this study was to produce a new mouse line that displays both obesity and intestinal tumorigenesis. To this end we have generated double mutant C57BLKS-*mLepr*$^{db/db}$; *Apc*$^{1638N/+}$ mice. Homozygous *db/db* mice carry a missense mutation resulting in premature termination of the intracellular domain of the long form of the leptin receptor associated with a complex phenotype of obesity and diabetes mellitus. Mutant *Apc*$^{1638N/+}$ mice progressively develop intestinal neoplasia and serve as a suitable model for human adenomatous polyposis syndrome.

Heterozygous *Apc*$^{1638N/+}$ and C57BLKS-*mLepr*$^{db/+}$ mice were crossed. Double heterozygous C57BLKS-*mLepr*$^{db/+}$; *Apc*$^{1638N/+}$ mice were then intercrossed to generate the obese double mutant offspring used in this study. All animals were euthanized at 6 months of age. The gastrointestinal tract was removed and paraffin sections of all regions were stained with hematoxylin and eosin to identify preneoplastic and neoplastic lesions.

The main findings were (i) homozygous *db*/*db* mice did not develop gastrointestinal neoplasia, (ii) the introduction of the db mutation into the mutant *Apc*$^{1638N/+}$ background not only enhanced *Apc*-driven development of small intestinal polyps but also induced the formation of gastric and colonic tumors. It is noteworthy that these tumors do not develop in single mutant *Apc*$^{1638N/+}$ mice at the age of 6 months age and are only infrequently observed in older *Apc*$^{1638N/+}$ mice. All tumors were adenomas. No gastrointestinal tumors were found in wild-type littermates.

These findings indicate that the hormonal and metabolic background of the *db*/*db* mouse exacerbates gastrointestinal neoplasia in the presence of a pre-existing *Apc* mutation and provide evidence of a mechanistic link between obesity and colorectal cancer.

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SMALL RENAL TUMOURS: SURGICAL CONSERVATIVE APPROACH

A. Lapini, A. Minervini and M. Carini

Department of Urology, University of Florence, Italy

Introduction: The widespread use of ultrasonography has led to an increasing number of early detected/incidentally found small renal masses, which are potentially suitable for nephron-sparing surgery (NSS). Presently, NSS is the accepted standard treatment in patients with small renal tumors and a normal contralateral kidney. We routinely perform the tumor enucleation (TE) technique, which consists of excising the tumor by blunt dissection without a visible rim of normal parenchyma. We report our experience with tumor enucleation in 303 patients. *Patients and Methods:* Between 1986 and 2004 303 patients with sporadic unilaterally RCC underwent NSS by tumor enucleation. In 232 patients, preoperative imaging evaluation showed renal mass <4 cm in greatest dimension,pathological evaluation confirm pT1a Renal cell cancer. In 71 cases the tumors were >4 cm but <7 cm in greatest dimension, and pathological review according to the 2002 TNM classification showed that 42% of the tumors (30 of 71) were pT1a, 44% (31 of 71) were pT1b and 14% (10 of 71) were pT3a. *Results: ≤4 cm RCC*. The mean (median, range) follow-up was 76 (61, 12-225) months. The 5- and 10-year cancer-specific survival were 96.7% and 94.7%, respectively. The 5- and 10-year progression-free survival were 96% and 94%, respectively. Overall, 13 (6.4%) patients had disease progression, three of whom had local recurrences alone (1.5%). *4-7 cm RCC*. The mean follow-up was 74 months (median 51, range 12 to 225). Five and 8-year cancer specific survival was 85.1% and 81.6%, respectively. Five-year cancer specific survival in patients with pT1a (4 cm), pT1b and pT3a disease was 95.7%, 83.3% and 58.3%, respectively). Overall 10 patients experienced progressive disease (14.9%), of whom 3 had local recurrence (4.5%) alone or local recurrence associated with distant metastases. *Discussion:* To avoid the risk of local recurrence, the excision of a minimal and visible margin of normal-appearing parenchyma around the tumor is considered the standard surgical technique of NSS. Recent reports concluded that the width of the resection margins does not correlate with disease progression and that if the tumor is completely excised, the margin size is irrelevant, thus providing an intriguing insight into the possibility of bluntly excising the tumor, such as a tumor enucleation. *Conclusion:* Tumor enucleation is a safe and acceptable nephron-sparing treatment that provides excellent long-term local control and survival rates.

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CURRENT CHALLENGES IN TUMOR IMMUNOLOGY: BETTER UNDERSTANDING TUMOR/IMMUNE SYSTEM CROSS-TALKS

Réjean Lapointe, Jessica Godin-Ethier, Marie-Andrée Forget, Sandy Pelletier, Simon Turcotte and Stéphanie Lepage

Tumour immunology laboratory, Institut du cancer de Montréal, Centre de recherche, Centre hospitalier de l'Université de Montréal (CRCHUM) Hôpital Notre-Dame, Montréal, Québec, Canada

Cancer is a major challenge in our society; although early surgery, radiotherapy and chemotherapy are effective and recognized, cancer is still a major killer. Alternative strategies are needed to improve outcomes for affected patients. Cancer immunotherapy is based on prompting the immune system to specifically recognize and kill tumours. Tumour regression mediated by an immunological response has been demonstrated in multiple clinical trials. However, we have to face a hard reality: the first generation of human cancer immunotherapies, especially that emerging from animal models, is effective in a limited number of patients. Tumours may negatively influence the cancer-specific immune response, and the search for major mechanisms mediating immune tolerance to tumours has been intense. The mechanisms include a lack of T-cell help, inadequate T lymphocyte functions (proliferation, lysis, *etc*.), decreased TCR signalling, and local production of immunosuppressive factors with many others (regulatory T-cells [Treg], MHC expression by tumours, *etc*.) also being proposed. These

mechanisms will be briefly reviewed and our recent data on tumour-influenced immunological environments will be presented. Our recent immunotherapeutic strategies to optimize antigenic presentation will also be described. Cancer immunotherapy can work, and efficiency could be increased once we have better control of negative immune regulators from tumours, and develop more appropriate ways of triggering a specific immune response.

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GENETIC AND PROTEOMIC PROFILING OF PROSTATE CANCER PROGRESSION

S.E.T. Larkin[1], I.A. Cree[2], S. Holmes[3], T. Walker[2] and C.L. Hastie[1]

[1]School of Pharmacy and Biomedical Sciences, University of Portsmouth;
[2]Department of Histopathology, Queen Alexandra Hospital, Portsmouth;
[3]Department of Urology, St Mary's Hospital, Portsmouth, UK

Advanced metastatic prostate cancer (PCa) is usually treated with hormone therapy, though this is rarely curative. In younger men, with organ-confined disease, radical prostatectomy is carried out to eliminate the risk of metastasis in later years. This procedure carries risks of impotence, urinary incontinence and even death.

The aims of this project, therefore, were to investigate the genetic and proteomic profiles of PCa in patients with varying stage and grade of the disease to identify prospective predictive markers of clinically significant PCa.

To identify prognostic markers in both studies, samples were split into two groups: Gleason grade ≥7 and Gleason grade <7. Using Taqman qPCR, a pilot study of gene expression in FFPE prostate tissue (46 samples) was examined. Ninety-six genes of interest were identified from literature reviews and microarray data mining. Results of this study show a significant difference ($p<0.05$) in at least 10 of the genes analysed. Three of these are significant to $p<0.025$. These three genes are the first to be analysed further by immunostaining of tissue microarrays to analyse protein expression, the results of which will be presented at this meeting. Additionally, a novel 2D gel electrophoresis method, encompassing liquid phase IEF followed by large format SDS PAGE, and mass spectrometry has enabled the study of serum proteomic profiles. Study of the presence and absence of proteins between the two patient groups (8 with Gleason <7, 18 with Gleason ≥7) has revealed 6 proteins of interest. Mass spectrometry analysis has identified 4 of these proteins and further analysis of two of the most interesting is currently being undertaken. Western blotting data of the expression of these proteins will be presented at this meeting.

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MANNOSE-6-PHOSPHATE/INSULIN-LIKE GROWTH FACTOR-II RECEPTOR IN HUMAN MELANOMA CELLS: EFFECT OF LIGANDS AND ANTIBODIES ON THE RECEPTOR EXPRESSION

Friedemann Laube

Institute of Physiological Chemistry, Martin Luther University Halle-Wittenberg, D-06097 Halle, Germany

The Mannose-6-phosphate/insulin-like growth factor II receptor (M6P/IGF-II) R is a multifunctional transmembrane glycoprotein that induces cellular responses in special cells. Previous attempts have given controversial results about the signal transduction capability of the receptor. However, more recent studies have shown the capability of M6P/IGF-II R to initiate transmembrane signalling (1).

Human melanoma cells were used to detect the cell surface expression of M6P/IGF-II R by immunoluminescence. The incubation of melanoma cells with M6P (5 mM) caused an increase of the receptor signal of ~50%. Pre-incubation of M6P-non-stimulated cells with cycloheximide (CHI, 10 μg/ml) reduced the receptor expression of ~15%, whereas actinomycin D (Act-D, 5 μg/ml) did not. But 30 min pre-incubation of cells with the inhibitors following M6P stimulation in the presence of Act-D or CHI caused a reduction of transcription of 27% and a reduction of receptor protein synthesis of 31%, respectively. Two monoclonal antibodies (mAb 2G11 and MEM-238) and a polyclonal Ab were used for the detection of the receptor. 2G11 was able to mimic the M6P ligand effect but MEM-238 did not. The signal released by the 2G11-stimulated receptor up-regulation was completely quenched by masking the receptor-bound 2G11 with anti-mouse IgG. The additive signal intensity detected with the combination of M6P and 2G11 and the failure of 2G11 to compete with the M6P action suggests that both effectors have different binding sites on the receptor. Unlike 2G11, the mAb MEM-238 prevented the M6P effect on receptor expression, confirming partially overlapping binding epitopes of both effectors on the extracellular receptor domains 1-**3**. MEM-238 recognizes an epitope between domains 2 and 5. Either the M6P binding site between domains 1-**3** is essential for its stimulating effect or both M6P binding sites (domains 1-**3** and 7-**9**) have to be occupied by M6P for the receptor up-regulation. It was possible to show an inhibiting effect of brefeldin A on the vesicular transport of the receptor protein to the plasma membrane and an activating effect of forskolin on the receptor secretion or exocytosis, respectively. Results obtained on the dynamic behaviour of the M6P/IGF-II R on melanoma cells support recent data demonstrating its capability of signal transduction.

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PATHOLOGICAL ASPECTS OF HETEROGENEITY OF GLIOBLASTOMA MULTIFORME

Libero Lauriola

Institute of Pathology, Faculty of Medicine, Catholic University of the Sacred Heart, Rome, Italy

Glioblastoma is the most malignant among brain tumours with astrocytic differentiation. It is also the most frequent glioma and affects primarily the cerebral hemispheres of adults, with a peak incidence in the fifth and sixth decades.

The definition of glioblastoma, recalling an embryonal or poorly differentiated tumour, echoes the uncertainty still present in the 1979 WHO classification. Glioblastoma is now considered as derived by the neoplastic transformation of mature astrocytes or astroglial precursors. The tumour is characterized by a high cell heterogeneity and pleomorphism, that complicates the description of all the tissue patterns and morphologic variations of the neoplastic cells; however, scattered differentiated astrocytic neoplastic cells are also frequently present. In addition to the more common pattern, glioblastomas can be composed of small uniform cells, whereas other tumours can be prevalently composed of pleomorphic giant cells. Other variants are sarcoma-like glioblastoma, glioblastoma with carcinoma-like features and those containing metaplastic bone or cartilage. Vascular proliferation, with features of 'microvascular' or 'endothelial' proliferation, and necrosis, consisting in large areas of tissue destruction or small foci of 'pseudopalisading' are cardinal features of glioblastoma.

The finding of large areas of well-differentiated astrocytic neoplastic cells coexisting with glioblastoma in cases of long clinical duration led to the conceptual distinction, firstly made by Scherer in 1940, between 'primary' and 'secondary' glioblastoma. Primary and secondary glioblastomas are now considered distinct subtypes, affecting different age groups and developing through different genetic pathways. Interestingly, they show different expression of some molecules and different responses to therapy. The diagnosis of secondary glioblastoma requires clinical and histological evidence of derivation from a low grade astrocytoma (WHO grade II) or anaplastic astrocytoma (WHO grade III). Primary glioblastomas pursue a short clinical course and do not show histological evidence of a less malignant component. These glioblastoma subtypes also differ at the molecular level. Ohgaki *et al*. (Cancer Res, *64*: 6892-6899, 2004) on a large population-based study demonstrated that TP53 mutations are the most frequent genetic alteration in secondary glioblastoma and are also present in the precursor low-grade or anaplastic astrocytoma. On the other hand, EGFR amplification, a possible target for therapy, and PTEN mutation were detected more frequently in primary (36% and 25%, respectively) but very rarely in secondary glioblastomas.

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THE EMERGING SIGNALING VRK1 PATHWAY AND ITS IMPLICATIONS IN PROLIFERATION AND DNA DAMAGE RESPONSE

Pedro A. Lazo, Alberto Valbuena, Marta Sanz-García and Inmaculada López-Sánchez

Instituto de Biología Molecular y Celular del Cáncer, Campus Miguel de Unamuno, CSIC-Universidad de Salamanca, 37007 Salamanca, Spain

The VRK Ser-Thr kinases are a new subgroup in the human kinome with two active members with a conserved catalytic domain and unrelated regulatory domains in their *C*-terminus. Human VRK1 is the better known member. *VRK1* gene expression is regulated by entry and exit in the cell cycle. The kinase activity is regulated by phosphorylation by growth factors. Furthermore, induction of DNA damage by UV light, doxorubicin, or etoposide induces an additional phosphorylation that further raises the kinase activity. VRK1 is mostly located in the nucleus, particularly in proliferating cells. Among its targets are p53, c-Jun and ATF2. VRK1 specifically phosphorylates p53 in Thr18 preventing its interaction with Hdm2; thus stabilizing p53 and leading to its accumulation and induction of growth arrest. This arrest would be permanent unless an autoregulatory mechanism to eliminate VRK1 was functional. This mechanism has been identified as being regulated by a transcriptionally active p53, and its structure is similar to that of the p53-Hdm2 autoregulatory loop. Active p53 induces a gene, *DRAM*, that targets p53 for its proteolytic down-regulation *via* the lysosomal pathway; VRK1 requires an inducible proteolytic degradation because it is a very stable protein with a half-life of several days. The existence of this pathway predicts that in tumors with *p53* mutations, there should be an accumulation of VRK1 protein. This was confirmed in a series of lung carcinomas, where VRK1 accumulates if they harbor a *p53* mutation. A similar finding has been detected in breast cancer.

The knock-down of VRK1 by siRNA results in a block in cell cycle progression, due to the contribution of *VRK1* as an early gene, expressed at the same time as *MYC* and *FOS* oncogenes and required for cyclin D1 expression. VRK1 loss is accompanied by a loss in phosphorylation of RB suggesting it is required very early in cell cycle progression, probably in the G0 exit to G1 and before the restriction point. This loss is accompanied by accumulation of cell cycle inhibitors, such as p27, consistent with the cell cycle block. In human head and neck squamous cell carcinomas, VRK1 positively correlates with proliferation markers such as Ki 67, CDK2, cdc2, topoisomerase II, survivin and cyclins. These correlations

indicate a role early in the cycle. However, VRK1 can play additional roles in proliferating cells, such as participating in responses to DNA damage.

VRK1 activity can also be regulated by interaction with other proteins. VRK1 interaction with nuclear Ran regulates VRK1 activity. Interaction with RanGDP inhibits VRK1 kinase activity, but the interaction with RanGTP recovers the activity. RanGDP inhibits the kinase activity of VRK1 thus preventing the phosphorylation of Histone H3 in Thr3 and Ser10, required for chromatin condensation during mitosis. In this way, an asymmetric distribution of VRK1 activity in the nucleus can be achieved.

Early during mitosis it is necessary to fragment the Golgi apparatus in order for it to be redistributed in daughter cells. VRK1 is a necessary step required for Golgi fragmentation acting downstream of MEK1 and PLK3. VRK1 is a direct phosphorylation target of PLK3.

VRK1 is a novel kinase that participates in several processes related to cell cycle progression.

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PROTEINPROFILING FOR IDENTIFICATION OF BREAST CANCER BIOMARKERS IN TEAR FLUID AND SERUM

A. Lebrecht, D. Boehm, H. Koelbl and F. Grus

Dept. of Gynecology and Obstetrics, Johannes Gutenberg-University, Mainz, Germany

Introduction: In patients with breast cancer early diagnosis is important for survival. Actually mammography is the best available method. Although sensitivity is high, specificity is relatively low. New biomarkers are needed to improve the early detection of breast cancer. The aim of the study was to generate a protein biomarker profile in tear fluid and serum for breast cancer patients. We used this established biomarker profile subsequent to discriminate between cancer patients and healthy controls. *Methods:* We screened for potential biomarkers in tear fluid and serum 60 women with breast cancer (CA) and 60 healthy women (CTRL), matched to the age. Blood samples and tear fluid were drawn prior to surgery. We used SELDI – TOF – MS (Surface Enhanced Laser Desorption Ionisation – Time Of Flight – Mass Spectroscopy) for protein profiling. Three different active surfaces of the protein chips were used: cationic exchanger(CM-10), hydrophobe surface (H50) and metal ionic affinity surface with different binding properties. The chips were read by mass spectroscopy and the analyses were done by multivariate statistics. *Results:* In a pilot study with 20 patients we found complex protein and peptide distributions on all three surfaces. We identified the main proteins in tear fluid, like lysozyme and lipocalin. Between breast cancer patients and healthy controls we found statistically significant differences in the protein profiling ($p<0.001$). Second we used SELDI-TOF-MS for protein profiling with two different active surfaces of the protein chips, a cationic exchanger (CM-10), and a reverse-phase surface (H50) in 100 patients. The data were analyzed by multivariate statistical techniques and artificial neural networks. Both, in tear fluid and in serum, we could generate statistically significant biomarker panels ($p<0.0005$). The diagnostic pattern could differentiate CA from CRTL with a specificity and sensitivity of about 70% in tear fluid and a specificity of 77% and a sensitivity of 85% in serum. *Conclusion:* It was successful to generate biomarker panels in tear fluid and blood to discriminate breast cancer patients and healthy women. With the help of neural networks these panels show a respectable sensitivity and specificity. The protein chip technology could greatly facilitate the discovery of new and better biomarkers. It is a promising approach to analyse a high number of patients. Analysing tear fluid may be useful for high-throughput biomarker discovery.

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INITIAL TUMOR GROWTH AND TUMOR INVASION ARE MEDIATED BY PDGF-BB AND VEGF-A ACTIVATED FIBROBLASTS

Wiltrud Lederle[1,2] and Margareta M. Mueller[1]

[1]Group: Tumor and Microenvironment (A101), German Cancer Research Center (DKFZ), 69120 Heidelberg; [2]Experimental Molecular Imaging, University of Aachen (RWTH), Germany

Stromal fibroblasts that are activated by tumor-derived cytokines play a crucial role for tumor growth and invasion. In the HaCaT-model of skin carcinogenesis, we identified PDGF-BB and VEGF-A activated fibroblasts as key mediators driving initial tumor growth and tumor invasion. PDGF-BB induces the conversion of immortal non tumorigenic HaCaT cells to benign tumor growth by differentially regulating cytokine expression in stromal fibroblasts, by promoting their differentiation into myofibroblasts and by enhancing pericyte recruitment. De novo expression of murine VEGF-164 induces malignant and invasive tumor growth of the before non tumorigenic HaCaT cells, associated with a strong and ongoing activation of myofibroblasts. However, the VEGF-induced tumors are ulcerated with a disorganized epithelium that is interrupted by lacunae with limited basement membrane and endothelial cell coverage. Additionally, vessel maturation is strongly impaired. Tumor and vessel micromorphology are markedly improved by the combined expression of PDGF-BB and VEGF-A, leading to a similar tumor growth but more compact tumors with a mature functional tumor vasculature. Invasive growth of the tumor cells is dependent on the presence of fibroblasts and is mediated by their VEGF and PDGF induced MMP-expression.

Specifically the expression of MMP-13 by activated fibroblasts is essential for ongoing angiogenesis and invasion of malignant skin SCCs. These data highlight the importance of cytokine activated fibroblasts in promoting tumor growth and progression and emphasize the importance of interfering with the tumor-fibroblast interactions for an efficient tumor therapy.

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TUMOR-LOCALIZED LIGATION OF CD3 AND CD28 WITH SYSTEMIC REGULATORY T-CELL DEPLETION INDUCES POTENT INNATE AND ADAPTIVE ANTITUMOR RESPONSES

Chien-Hsin Lee, Yi-Shen Chiang, Shih-En Chang, Chin-Liew Chang, Bing-Mae Cheng and Steve R. Roffler

Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC

Tumor-localized activation of immune cells by membrane-tethered antibodies is currently under investigation to treat poorly immunogenic tumors. Here, we investigated the mechanism of antitumor immunity elicited by tumor-located expression of a membrane-tethered anti-CD3 single-chain antibody (CD3L). Expression of CD3L and CD86 on poorly immunogenic B16 melanoma cells (B16/3L86 cells) activated naïve T-cells, suppressed tumor growth in subcutaneous, peritoneal and metastasis models, and protected mice from rechallenge with B16 melanoma cells. However, *in vivo* antitumor activity against primary B16/3L86 tumors unexpectedly depended on NK cells rather than $CD4^+$ or $CD8^+$ T-cells. Treatment of mice with low-dose cyclophosphamide, or anti-CD25 antibody to deplete regulatory T-cells unmasked latent T-cell antitumor activity; the number of activated $CD8^+$ T-cells in tumors increased and B16/3L86 tumors were completely rejected in a $CD8^+$ and $CD4^+$ T-cell-dependent fashion. Furthermore, fibroblasts expressing CD3L and CD86 suppressed growth of neighboring B16 cancer cells *in vivo* and direct injection of adenoviral vectors expressing CD3L and CD86 or CD3L and a membrane-tethered anti-CD28 antibody significantly suppressed B16 tumor growth.

Taken together, our results show that tumor-located ligation of CD3 and CD28 can activate both innate (NK cell) and adaptive ($CD4^+$ and $CD8^+$ T-cell) responses to create a tumor-destructive environment to control tumor growth, but modulation of Tregs is necessary to unmask local adaptive antitumor responses. Depletion of Tregs and pharmacological stimulation of NK cell activity are potential avenues to increase efficacy of therapies that activate lymphocytes *via* CD3, including bispecific antibody therapy of cancer.

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MATRIX METALLOPROTEINASE-9 INDUCES EPITHELIAL-TO-MESENCHYMAL TRANSITION IN A HIGHLY INVASIVE A431 TUMOR CELL SUB-LINE

Chun-Yu Lin[1], Yung-Sheng Lin[1], Lung-Ta Lee[2], Ping-Ping H. Lee[3], Wen-Te Kao[1], Pei-Hsun Tsai[1], Ferng-Chun Ke[4], Jiuan-Jiuan Hwang[5] and Ming-Ting Lee[1,3]

Institutes of [1]Biochemical Science and [4]Molecular and Cellular Biology, National Taiwan University, Taiwan; [2]Department of Nutrition and Health, ToKo University, Taiwan; [3]Institute of Biological Chemistry, Academia Sinica, Taiwan; National Taiwan University, Taiwan; [5]Institute of Physiology, National Yang Ming University, Taipei, Taiwan, ROC

Recently, highly invasive tumor cell lines (designated A431-I, -II and -III) derived from parental A431 tumor cells (A431-P) were isolated in our laboratory by three successive passages through a Boyden chamber with matrigel-coated membrane support. The invasive potential and the activity of secreted MMP-9 of each subline increased significantly compared to the A431-P (A431-III > A431-II > A431-I) as evidenced by the *in vitro* invasion assay, gelatin zymography and immunoblotting analyses. RT-PCR results also revealed the elevated expression of MMP-9 in A431-III. We further characterized the A431-III subline and found that these cells exhibited a greater potential for attachment and spreading on fibronectin-coated substratum and for migration. The A431-III cells displayed multiple cytoplasmic extensions with focal contacts (vinculin-positive staining) during cell spreading. Our results indicate that an increase in MMP-9 secretion could disrupt the E-cadherin complex of proteins and lead to the loss of cell adhesion. It is well known that epithelial-to-mesenchymal transition (EMT) can result in the loss of E-cadherin. Therefore, we decided to investigate whether MMP-9 could induce EMT in a highly invasive A431-III tumor cell sub-line. In this study, we noticed a strong multigene signature indicative of EMT as identified by microarray analysis. The observation of the increase of EMT marker proteins was further supported by immunoblotting, including N-cadherin, vimentin, fibronectin, MMP-3, MMP-9, Snail1, Twist1 and TGF-β2. Taken together, these results demonstrate that the greater invasion potential exhibited by the highly invasive. In conclusion, the invasiveness of the A431-III subline is likely attributed to an increased ability for attachment, spreading and migration, as well as increased MMP activity. That action of MMP-9 on cancer cells can induce EMT provides insight into novel therapeutic targets. A431-P and the highly invasive A431-III subline could be an excellent model for studying the mechanism of cancer metastasis.

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DNA REPAIR PROTEINS ASSOCIATED WITH PHOTOFRIN RESISTANCE IN U87 GLIOBLASTOMA CELL LINE

S.Y. Lee, S.P. Yip and S.S.T. To

Dept. of Health Technology and Informatics, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, China

Background: Photodynamic therapy (PDT) is a new modality of cancer therapy which relies on the use of light and the preferential accumulation of photosensitisers in neoplastic tissues to kill cancerous cells. PDT is suitable as an adjunct therapy for glioma. However, the failure of PDT to have any effect has been reported in some studies, and the underlying cellular mechanisms leading to failure are still unknown. DNA repair mechanisms may account for reported PDT failures. During the course of PDT, reactive oxygen species generated by the photosensitiser may interact with DNA, causing chromosomal damage and mutations. This in turn leads to apoptosis and necrosis in the cancerous cells. However, if the DNA lesions caused by PDT are repaired, the cells may survive the damage induced by PDT. We hypothesise that if the expression of DNA repair proteins is high in glioma cells, the cells can efficiently repair the DNA damage caused by PDT. Consequently, these glioma cells become more resistant to PDT and therefore are not susceptible to the actions of PDT. *Objectives:* To investigate the role of DNA repair mechanisms in glioma cells resistant to Photofrin-based PDT. *Methods:* The expression levels of DNA repair messenger ribonucleic acids (mRNA) and DNA repair proteins in different grades of Photofrin-PDT-resistant U87 glioma cells were examined using quantitative real-time polymerase chain reaction and Western blotting. The mRNA expression data were further analysed by one-way analysis of variance followed by Tukey post-test. Six specific genes representative of six DNA repair mechanisms were examined. *Results:* Four of the DNA repair genes investigated have comparable mRNA expression levels between the controls and Photofrin-PDT resistant U87 glioma cells. The two exceptions were the *ALKBH2* (alkB, alkylation repair homolog 2 (*E. coli*)) gene of DNA damage reversal and the *REV1* (REV1 homolog (*S. cerevisiae*)) gene of translesion synthesis. The mRNA transcripts of these two genes were expressed at a significantly higher level in Photofrin-treated cells ($p<0.01$) when compared with the control cells. Increased expression was observed from 30 minutes to 48 hours post-treatment with Photofrin. These results were also confirmed at the protein level by Western blotting. This suggested that induced expression in both genes occurred immediately in treated cells after irradiation with the light source. *Conclusion:* Since high mRNA and protein expression of *ALKBH2* and *REV1* were observed in Photofrin-PDT resistant glioma cells but not in control cells, the results indicate that both DNA damage reversal and translesion synthesis mechanisms may play important roles in Photofrin resistance. High expression of REV1 also indicates that much DNA damage is produced during Photofrin-PDT and cannot be repaired. Further work is in progress to investigate the relationship between the expression of DNA repair proteins and Photofrin resistance by using silent RNA, and to evaluate the types of DNA damage generated by Photofrin.

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RADIATION-INDUCED CHANGES IN THE MOUSE BRAIN PHOSPHOPROTEOME ANALYZED BY DIFFERENTIAL $^{16}O/^{18}O$ LABELING AND UPLC-MS/MS

Dominic Winter[1], Joerg Seidler[1], Yael Ziv[2], Yosef Shiloh[2], and Wolf D. Lehmann[1]

[1]Molecular Structure Analysis, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany;
[2]Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Differential $^{16}O/^{18}O$ proteolytic labeling (1) is a versatile method in analytical proteomics for relative quantitification of two samples. The latest version of MASCOT contains a tool for relative quantification within pairs of $^{16}O/^{18}O$ labeled peptides (2). Experimental and procedural parameters for LC-MS/MS analysis were set-up, which allowed an accurate quantification using this tool. This is demonstrated using standard peptide mixtures and ovalbumin digest mixtures. The method was then for the first time applied to quantitative proteomic analyses in a complex sample. Quantitative changes in the mouse phosphoproteome induced by irradiation-induced DNA damage before and after treatment with a dose of 10 Gy were investigated.

For this purpose, mouse brain proteome samples were prepared and digested with trypsin, using ^{16}O water for the treated sample and ^{18}O water for the control sample. After combination of a pair of samples, fractionation of the complex peptide mixture by mixed-bed ion-exchange chromatography was performed. Each fraction was subjected to phosphopeptide enrichment by IMAC and both the flow-through fractions and the phosphopeptide fractions were analyzed separately by nanoUPLC-MS/MS. For the quantitative analysis, all data were combined into a single file and analyzed by MASCOT. As a result, 342 proteins were identified. In the analysis of phosphorylation sites, 174 phosphopeptides were identified, of which 24 were up- or down-regulated by more than a factor of 2.

Several up-regulated phosphorylation sites observed stem from proteins known to be involved in signaling cascades or DNA-binding. These include ERK, MAP tau, MAP 2, MAP kinase-activating death domain, p130Cas-associated protein, R3H domain containing protein, etc. The results are discussed in relation to the involvment of known signaling cascades in the response to radiation induced DNA damage. It is concluded, that the $^{16}O/^{18}O$ method is suitable for monitoring quantitative changes in the phosphoproteome of complex protein samples.

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ALTERATION OF CHEMOTHERAPEUTIC EFFECT OF ANTICANCER AGENTS LINKED TO HYPOXIA OF MDR AND NON-MDR LOVO COLON CARCINOMA CELLS

Isabelle Lelong-Rebel[1], Erwan Pencreach[2], Gérard Rebel[1] and Jean-Pierre Bergerat[1]

[1]UPRES EA 3430-ULP/CNRS – IRCAD - Hôpital Civil, BP 426, F-67091 Strasbourg;
[2]INSERM U682, 3 avenue Molière, F-67200 Strasbourg France

Culture of cancer cells is widely used for testing antiproliferative drug response in cancer research. Cell resistance against antineoplastic agents often results from mobilization of various factors, the modulation of which is linked to the culture conditions. Most of the protocols utilize cells growing in [air+5-10%CO_2] atmosphere thus achieving a 19-20%O_2 level. Under these conditions, cells are growing in hyperoxic environment, as the highest physiological partial oxygen pressure (pO_2) found in mammals is 16% (*e.g.* blood from hepatocyte artery). Most of the cells within solid tumors are exposed to a low pO_2, this hypoxic condition (*e.g.* 0.1-5%O_2) being linked to their location within the tumor, the tumor compaction, and the presence or absence of lymphovascular tumor emboli. This low pO_2 affects notably cell physiology, the level of ROS generated and the cell growth in response to various drugs.

In this study we addressed the variation of the pharmacological response of MDR and non-MDR variants of LoVo colon carcinoma grown either in hypoxia (5%O_2) or in standard conditions (20%O_2), using a multigas incubator. We have tested the effect of pO_2 on the cytotoxicity of doxorubicin, cisplatin, 5-FU and combinations. Drugs were continuously present and proliferation was monitored every 3 day during 9 days. In the absence of drugs, all LoVo variants grew notably faster at 20%O_2 than at 5%O_2 [3X for MDR, 2X for non-MDR]. Moreover, respective sensitivities of both variants to doxorubicin and cisplatin were lowered by about 20% at 5%O_2 compared to 20%O_2. Non-MDR and MDR cells that were never exposed to the drug, showed a relative high resistance to 5-FU (IC_{50}: 0.36 μM; 0.55 μM, respectively) that was not affected by pO_2.

Our results underline the importance of evaluating the role of hypoxia on the cytotoxic effect of chemotherapeutic agents and on the proliferative status of cells. These data will be discussed taking into account the drug metabolism with respect to environmental oxygen pressure and cell culture conditions.

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SPATIAL REDISTRIBUTION OF P-GLYCOPROTEIN LINKED TO ESTRADIOL TREATMENT IN ENDOMETRIAL AND PROSTATIC CANCER CELLS

Isabelle Lelong-Rebel, Gérard Rebel and Jean-Pierre Bergerat

UPRES EA3430-ULP/CNRS-IRCAD Hôpital Civil, BP 426, F-67091 Strasbourg, France

Chemoresistance in oncology refers to complex and multifactorial process involving various cellular pathways. One of the most studied causes of multidrug resistance (MDR) is related to various ABC transporters being capable of overcoming cytotoxic xenobiotics *e.g.* anticancer agents aggression of the cells by mean of active efflux. Among these, P-glycoprotein (Pgp, ABCB1) is often representative of individual cell resistance particularly when overexpressed in the plasma membrane of multidrug resistant cancer cells. Although this pericellular location is related to "standard" MDR phenotype, intracellular location of such transporter(s) in numerous non-MDR tumor cells has also been described. It was hypothesized that this peculiar location could be in relation either with a primary defense mechanism *e.g.* involving cytosolic "scavenging" of the drugs or would be related to another physiological pathway, to date unknown. Being interested in MDR phenotype emergence in hormone-dependent cells, apart from cytotoxic drug challenge and selection, endometrial cell lines from our laboratory were screened for presence of P-glycoprotein in conjunction with estradiol treatment. Variants of Ishikawa endometrial carcinoma cell lines with differential panel of sensitivity towards 17-beta-estradiol (E2) and towards tamoxifen were studied. As E2 plays an important role in male physiology via the androgen receptor (AR), the prostatic LNCaP cell line was

also used in this study. All cell lines showed cytosolic location of P-glycoprotein in the absence of E2. Estrogen receptor (ER) was detected in all endometrial cell lines and underwent cytosol to nuclear location upon E2 treatment of the cells. ER was present in LNCaP cells as well. By far more intriguing was the overall spatial tissue redistribution of Pgp from a patchy-like to an islet-like pattern when Ishikawa monolayers were treated with E2. Emerging evidence exists to suggest that some of the pivotal cancer-related biological events depend on multicellular interactions between cell sub-populations that are subjected to inter-connective pathways via environmental stimulations.

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SIGNAL TRANSDUCTION AND MODULATION OF RADIOSENSITIVITY

Johan Lennartsson

Ludwig Institute for Cancer Research, Uppsala University, SE-751 24 Uppsala, Sweden

The emerging connections between growth factor-induced signal transduction and the DNA repair machinery will be described, stressing implications for radiotherapy. Furthermore, the prospect of developing targeting agents, which selectively deliver radioactivity to the tumor and at the same time radiosensitize tumor cells will be discussed. In addition, the possibility of increasing radiosensitivity by the use of low molecular weight compounds will be addressed.

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REGULATION OF MAP KINASE SIGNALING IN RESPONSE TO PDGF STIMULATION

Aleksandra Jurek, Carl-Henrik Heldin and Johan Lennartsson

Ludwig Institute for Cancer Research, Uppsala University, Sweden

The MAP kinase family is evolutionary conserved and mammalian cells contain several MAP kinase pathways, *e.g.* Erk1/2, p38, Jnk and Erk5. Several biological responses are controlled by MAP kinase activity and both the magnitude and kinetics of activation encode information for the cell on how to respond to the stimulus. A large fraction of human tumor cells have augmented MAP kinase activity, emphasizing the importance of these pathways in the development of cancer. Improper MAP kinase signaling can be caused by oncogenic activation of an upstream component such as Ras or loss of a negative regulator. This presentation will discuss the importance of the negative regulator MAP kinase phosphatase 3 in proper Erk1/2 activation in response to PDGF, as well as cross-talk between the Erk1/2 and Erk5 cascades. We have found that PDGF induces a rapid proteasomal degradation of MKP3, which is necessary for Erk1/2 activation to occur. Moreover, prolonged PDGF stimulation induces expression of the *mkp3* gene and subsequent increase in MKP3 protein level. The re-synthesis of MKP3 plays an important role in reducing Erk1/2 signaling at a later stage. Thus, MKP3 has a central role in two phases of signal transduction: first a rapid feed-forward mechanism and second a later feed-back loop. Furthermore, we have data suggesting that activation of Erk5 and Erk1/2 in response to PDGF negatively regulate each other in a reciprocal manner, providing an additional level of MAP kinase activity regulation.

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BEDSIDE TO BENCH: PROFILING BIOLOGICALLY BASED CAM PRODUCTS IN MALIGNANCY USING THE HERBAL COMPOUND ESSIAC AS A CASE STUDY

Blair J.N. Leonard

McMaster University, Hamilton, Canada

The use of biologically based complementary alternative therapies (CAM) in patients with malignancy is a well documented phenomenon of international scale, with different studies estimating use in 20-80% of cancer patients depending on the studied population. Biologically based CAM use in malignancy is not benign, with several *in vitro* and clinical studies documenting adverse interactions between common CAM therapies and conventional chemotherapeutic treatments. Despite widespread use and the establishment of evidence-based practice guidelines in oncology that call for further research into these therapies, many commonly used therapies remain largely unstudied.

An archetypal example of such pharmacological therapy is Essiac tea, a proprietary mixture of four herbal compounds that has been available without restriction for use in cancer patients for over 80 years. Several recent large studies of CAM use in oncology patients have confirmed Essiac as a popular choice, including documenting its concomitant use during both conventional radiation and chemotherapeutic management of malignancy. Despite this, rational and rigorous approaches adopting the scientific method to understand the pharmacological, as well as the pathophysiological properties of Essiac are lacking in the peer reviewed medical literature.

This presentation will outline the body of peer reviewed basic research involving Essiac that is currently available. Specific attention will be placed on the recent *in vivo* and *in vitro* series of experiments that investigated several alleged medicinal qualities of Essiac, as well as the potential for a harmful side-effect profile. Data derived from these investigations and others have identified pharmacological characteristics of Essiac that make it of interest for further

investigation: data that is freely available for access and appraisal by health care practitioners and their patients for informed choice. This last point underscores the overriding theme of the presentation, as previous studies identified that patients derive much knowledge on which they base decisions about their CAM-related health care on unreliable, non-peer reviewed sources.

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A TWENTY YEAR JOURNEY TO UNDERSTANDING AND TREATMENT OF THE CHRONIC FATIGUE SYNDROME INCLUDING A LONGITUDINAL STUDY OF GROUPS A AND B CFS PATIENTS, 2000-2006

A. Martin Lerner

32804 Pierce St, Beverly Hills, MI 48025 USA

The Energy Index point score (EI), (copyright, Lerner AM and Deeter RG, 1999), (0-10) is a simple reliable metric easily evaluating the functional capacity at each CFS patient-physician visit. A hanging sign in the examining room, with physician and patient together, is used. Validation of the EI was carried out using two methods: a) 20 CFS patients and 22 healthy adults, matched for sex, age, place and time; EI, CFS=3.6; EI, healthy adults=9.9, $p \leq 0.0001$, and b) 55 CFS patients evaluated at the same time by the EI and Fatigue Severity Score, correlation 0.67, $p=0.0066$. Improvement to disappearance of CFS symptoms correlate with an increasing EI.

The validated Energy Index (EI) point score (1-10) was calculated for each CFS patient every 3 months at physician visits. A CFS patient has an EI≤5. A CFS patient with an EI of 0 is bedridden; a CFS diagnosis is no longer present at an EI>5. The EI effect size is 0.25, a medium effect size is 0.5. A large effect size is >0.8. Administrations of antiviral drugs were given within a defined pharmacokinetic therapeutic window.

Eighteen CFS patients with elevated serum IgG serum antibody titers to cytomegalovirus (HCMV) were treated with intravenous ganciclovir 5 mg/kg *q* 12 h for 30 days. At evaluations, 24 weeks later, 13 patients (72%) returned to their premorbid healthy states (Infectious Diseases in Clinical Practice, *6*: 110-117, 1997). In a second study, 25 CFS patients with elevated serum antibody titers to Epstein-Barr virus (EBV), early antigen (Diffuse) and/or EBV, viral capsid antigen (VCA, IgM) were treated with valacyclovir (14.6 mg/kg *po q* 6 h) for 6 months. This valacyclovir dose achieved serum acyclovir C_{max}>7 μm and high antiviral activity *versus* EBV (ID_{50}, 4.4-13.3 μm), but no antiviral activity *versus* HCMV. The CFS patients EI functional capacity as well as EBV and HCMV serum antibody titers were again assessed after 1, 3 and 6 months of valacyclovir. We concluded that the 16 CFS patients with EBV persistent infection (EBV single-virus subset) improved after 6 months, but 9 CFS patients with elevated serum antibody titers to "both" EBV and HCMV did not benefit from valacyclovir (Drugs of Today, *38*: 549-561, 2002). With this guidance, a randomized double blinded controlled 6 month study of EBV subset single virus (no HCMV serum antibody) showed an EI rise after 6 months of +1.12 units (122 kcal/day), in the valacyclovir group while the placebo group improved +0.42 units (65 kcal/day), In Vivo *21*: 707-714, 2007.

The current inclusive CFS data (May 1, 2001-December 31, 2007) regardless of duration of CFS illness from this treatment center of 201 CFS patients include 5,700 visits and 44,000 fields of information which reveal demographic and epidemiologic data, 156 (77.6%) female; 45 (22.4%) male. The mean age of CFS patients is 45.2 years, BMI 26.4 Kg/m^2. These 201 CFS patients are two distinct groups with similar demographics; (A) CFS Herpesvirus Illness (EBV, HHV6, HCMV) with no co-infections, and Group (B) CFS Herpesvirus Illness (EBV, HCMV, HHV6) "with" mimicking, co-infections, both A group and B group meeting international criteria for diagnosis of CFS. (Fukuda, Ann Intern Med, *121*: 953-959, 1994). The major co-infections of Group B are Lyme disease, babesiosis, adult rheumatic fever.

The subsequent data here are those of CFS Group A who were ill an average of 5.2 years before receiving antiviral therapy. Data for CFS Group B are not included. There were 138 group A CFS patients, 104 females (75.4%) and 34 males (24.6%). The mean age was 46.4 years, BMI 26.7 Kg/m^2. Patients were further identified by the presence of elevated serum antibody titers to EBV, HCMV, or HHV6. CFS patients (>95%) had abnormal oscillating flat or inverted T-waves at 24 hr ECG monitor and abnormal cardiac wall motion at rest (11.5%) and stress (24.1%). Cardiac biopsies from CFS patients seen in 1997 showed a non-inflammatory cardiomyopathy with myofiber disarray, myofiber drop out, apoptosis, and cardiac replacement fibrosis.

Among the 138 Group A herpesvirus CFS patients there were single virus infections, EBV patients (27.5%); HCMV (13.8%); and HHV6 (1.4%). However, more commonly, each CFS patient was infected with several herpesviruses simultaneously: 79 patients with multiple herpesvirus infections (57.2%). There were EBV/HCMV co-infections (28.3%); EBV/HHV6 co-infections (10.9%); HCMV/HHV6 co-infections (5.1%); and EBV/HCMV/HHV6 co-infections (13.0%). Specific long-term pharmacokinetic therapy was administered to each patient until the EI point score reached 8, at which time, antiviral medicines were tapered, stopped, or continued, as appropriate with no change in the EI point score. The EI point score at 3 month intervals for the 6 years of the study was recorded. There were a mean of 46 EI patients at each 3 month time interval and 25 time intervals over the 6 year longitudinal study. The mean EI for the 138

CFS patients at baseline was 4.5. The mean final EI point score was 6.0, an increase of 1.5 EI units and, therefore, a large EI effect size change (Spearman's *p* nonparametric correlation test, Spearman's $p=0.562$, $p=0.0019$). These data indicate that specific long-term anti-herpesvirus pharmacokinetic administration of valacyclovir/valganciclovir provides long-term significant benefit to Group A CFS patients. There was no toxicity to this long-term antiviral therapy as given. For the evidence-based physician requiring placebo controlled double blinded trials for veritude, without recognition of the differences between Group A and Group B CFS patients, as defined here, it is likely that the evidence based trial may have falsely yielded "no benefit" from the antiviral therapy.

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CANCER AND INFLAMMATION: NEW INSIGHTS TO THE ROLE OF NF-KAPPAB AND COX-2 IN CARCINOGENESIS

Shahar Lev-Ari

Tel-Aviv Sourasky Medical Center, affiliated to the Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel

Cumulative experimental and epidemiologic evidence indicates that nuclear factor kappaB (NF-κB), a pro-inflammatory transcription factor, plays a significant role in carcinogenesis.

Activation of NF-κB was shown to regulate the synthesis of cyclooxygenase-2 (COX-2), as well as other signaling pathways which enhance tumor cell proliferation, angiogenesis, invasiveness and resistance to apoptosis-based tumor surveillance mechanisms and chemo-radiation therapies.

Research aimed at developing chemical and natural inhibitors that block NF-κB activation is currently flourishing. Several non-steroidal anti-inflammatory drugs (NSAID) and natural compounds, such as Aspirin and curcumin, have shown to inhibit NF-κB activity. Other agents targeting the IKK, and other upstream kinases involved in NF-κB activation such as bortezomib (Millennium Pharmaceuticals) and PS-1145 (Bristol-Myers) are under development. Several of these agents have shown anticancer activity in clinical or preclinical studies with acceptable safety profiles. Recent research has demonstrated that concurrent inhibition of NF-κB may increase chemo-radiotherapy efficacy. This could lead to novel therapeutic modalities in cancer therapy.

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IN VITRO AND *IN VIVO* EVALUATION OF [^{64}CU-NOTA-8-AOC-BBN(7-14)NH_2] RADIOPHARMACEUTICAL FOR PET IMAGING OF HUMAN BREAST AND PROSTATE CANCER TUMORS

Michael R. Lewis, Adam F. Prasanphanich, Lauren Retzloff, Stephanie R. Lane, Prasant K. Nanda, Gary L. Sieckman, Tammy L. Rold, Lixin Ma, Said D. Figueroa, Samantha V. Sublett, Timothy J. Hoffman and Charles J. Smith

The Radiopharmaceutical Sciences Institute, University of Missouri School of Medicine, Columbia, Missouri, 65211, USA

Introduction: Human prostate and breast cancers are known to express the gastrin-releasing peptide receptor (GRPr) in very high numbers. In this study, a derivative of bombesin (BBN) peptide, an agonist for the GRPr, has been complexed with Cu-64 radionuclide, making the conjugate a potential candidate for positron-emission tomography (PET) imaging and therapy of these human cancers. ^{64}Cu-NOTA-8-Aoc-BBN(7-14)NH_2 produced high-quality microPET images of GRPR-positive tumors in severely compromised immunodeficient (SCID) mice. *Methods:* Briefly, the unmetallated conjugate was synthesized by solid-phase peptide synthesis followed by manual conjugation of NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) bifunctional chelating agent. Radiolabeling with Cu-64 radionuclide was performed in buffered, aqueous solution (pH=7-7.5). The radiolabeled conjugate was assayed *in vitro* and *in vivo* T-47D (human breast) and PC-3 (human prostate) cell lines in order to determine its specificity and selectivity for the GRPr and its corresponding pharmacokinetic profile. *In vivo*, multimodal, molecular imaging *via* microPET/CT and microMRI was performed in tumor-bearing mice models bearing xenografted tumors. *Results:* The ^{64}Cu-NOTA-8-Aoc-BBN(7-14)NH_2 targeting vector was determined to specifically localize in GRPr-positive tissues. Accumulation of radioactivity was observed in the tumor in sufficient quantities to allow for identification of tumors in microPET imaging procedures. Uptake and retention of conjugate in T-47D xenografted tumors was determined to be 2.27±0.08%, 1.35±0.14%, and 0.28±0.07% ID/g (Percent Injected Dose Per Gram Tissue) at 1, 4, and 24 h. Uptake and retention of conjugate in PC-3 xenografted tumors was determined to be 3.58±0.70%, 1.64±0.17%, and 1.00±0.19% ID/g at 1, 4, and 24 h, respectively. *Conclusion:* The ^{64}Cu-NOTA-8-Aoc-BBN(7-14)NH_2 produced high quality microPET images. The pharmacokinetic profile of this conjugate justifies further preclinical evaluation of this new radiopharmaceutical as a potentially-useful diagnostic agent. Additionally, this new radiopharmaceutical serves as a good reference for modification and optimization of similar agents to maximize tumor uptake and minimize non-target accumulation of radioactivity in collateral tissues.

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RADIOPHARMACEUTICAL IMAGING AND THERAPY TARGETING THE *BCL-2* ONCOGENE IN B-CELL LYMPHOMA

Michael R. Lewis[1], Fang Jia[2], Ethan R. Balkin[2], Said Daibes Figueroa[1], Baghavathy S. Balaji[2], Fabio Gallazzi[3], Kimberly A. Statham[2] and Timothy J. Hoffman[1]

[1]Research, Harry S Truman Memorial Veterans' Hospital, Columbia, MO;
[2]Veterinary Medicine and Surgery, 3Molecular Biology, University of Missouri, Columbia, MO, USA

Introduction: The *B-cell lymphoma/leukemia-2* (*bcl-2*) oncogene is a dominant inhibitor of apoptosis, correlating with resistance to radiation and chemotherapy, high relapse rate, and poor survival in non-Hodgkin's B-cell lymphoma (NHL). NHL also expresses type 2 somatostatin receptors (SSTR2) in 87% of cases, making it attractive for delivery of intracellular tumor-targeting agents. *Methods:* A *bcl-2* antisense peptide nucleic acid (PNA) conjugated to a SSTR2-targeting peptide, anti-*bcl-2*-Tyr^3-octreotate, was evaluated for ^{111}In gamma scintigraphy and single photon emission computed tomography (SPECT), ^{64}Cu positron emission tomography (PET), and ^{177}Lu targeted radiotherapy (TRT) in NHL cells in culture, mouse models of human NHL, and dogs with spontaneously occurring NHL. SCID mice bearing bcl-2 mRNA-positive Mec-1 (n=3) or bcl-2 mRNA-negative Ramos (n=3) xenografts were used for ^{111}In microSPECT or ^{64}Cu microPET imaging. The ^{111}In conjugate was also used for gamma scintigraphy of canine NHL patients (n=15). The ^{177}Lu conjugate was evaluated *in vitro* for TRT in Mec-1 cells (n=3). *Results:* Incubation of Mec-1 cells with anti-*bcl-2*-Tyr3-octreotate showed a 51% decrease in bcl-2 protein synthesis, suggesting that the target mRNA function had been perturbed by a specific antisense effect. Both ^{111}In microSPECT and ^{64}Cu microPET could detect Mec-1 tumors, but not Ramos tumors, in SCID mice ($p<0.05$). Gamma scintigraphy demonstrated the utility of the ^{111}In conjugate for molecular staging of bcl-2 in canine NHL. *In vitro* Mec-1 cell studies showed that the ^{177}Lu conjugate had at least an additive effect on cell viability, compared to controls for targeted radioactivity and *bcl-2* antisense activity. *Conclusion:* 1) Imaging studies demonstrated that ^{111}In- and ^{64}Cu-anti-*bcl-2*-Tyr^3-octreotate were specific for bcl-2 mRNA-positive NHL xenografts. 2) Imaging of canine NHL established *bcl-2* expression as a clinical and molecular model relevant to human disease. 3) TRT studies of the ^{177}Lu conjugate in Mec-1 cells demonstrated down-regulation of *bcl-2* with radiation insult, creating a NHL therapy agent acting through two targeted anti-tumor mechanisms.

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CHARACTERIZATION OF BRIT1/MCPH1'S FUNCTION IN DNA DAMAGE RESPONSES USING A KNOCKOUT MOUSE MODEL

Yulong Liang, Hong Gao, Shiaw-Yih Lin, Pumin Zhang, Asha S. Multani, Sandy Chang, John A. Goss, Francis C. Brunicardi and Kaiyi Li

Department of Surgery, Baylor College of Medicine, Houston, TX, 77030, USA

The DNA damage-response pathway is essential for the maintenance of genomic stability; as a result, it acts as the barrier for tumorigenesis. Previous studies in cell culture demonstrated that Brit1 played critical roles in checkpoint control and DNA damage response. Aberrant expression of Brit1 was also identified in several types of human cancers. To address the physiological functions of Brit1 and the effect of its deficiency on tumorigenesis, we recently generated a Brit1 knockout (Brit1$^{-/-}$) mouse model and analyzed its *in vivo* function. We found that Brit1$^{-/-}$ mice can survive to adulthood but with growth retardation and low birth rate. Both Brit1$^{-/-}$ male and female mice were infertile with much smaller testes or ovaries. When analyzing Brit1$^{-/-}$ testes, we found that there were no spermatids and much fewer spermatocytes with meiosis dysregulation. Consistently, Brit1$^{-/-}$ spermatocytes exhibited DNA repair defects including reduced Rad51 and gamma-H2AX foci formation. In addition, both Brit1-defecient mice and MEFs (Mouse Embryonic Fibroblasts) were hypersensitive to gamma-irradiation. MEFs and T lymphocytes with Brit1$^{-/-}$ genotypes also exhibited severe chromosome aberrations. Notably, we found that, in contrast to their wild-type or heterozygote littermates, 45% of Brit1$^{-/-}$ mice with age one to two years old developed ovarian tumors. Moreover, tumor incidence in Brit1$^{-/-}$p53$^{-/-}$ double null mice is significant earlier than those in Brit1$^{+/+}$p53$^{-/-}$, indicating that loss of Brit1 enhances cancer susceptibility of p53 null mice. Together, the generation of Brit1 knockout mice provides convincing evidence that Brit1 is crucial for maintaining genomic stability *in vivo* to protect the hosts from both programmed and irradiation-induced DNA damages. In addition, our studies provide genetic validation of Brit1's role as a novel tumor suppressor gene.

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RECOMBINANT LEPTIN ADMINISTRATION IMPROVES EARLY ANGIOGENESIS IN FULL-THICKNESS SKIN FLAPS: AN EXPERIMENTAL STUDY

I.E. Liapakis[1], S. Anagnostoulis[2], A.J. Karayiannakis[2], D.P. Korkolis[3], M. Lambropoulou[4], B.C. Golematis[5] and C.E. Simopoulos[2]

[1]Department of Plastic and Reconstructive Surgery, University of Ghent, Ghent, Belgium;
[2]2d Department of Surgery and [4]Department of Pathology, Medical School, Democritus University of Thrace, Alexandroupolis;
[3]1st Department of Surgical Oncology, Hellenic Anticancer Institute, Aghios Savvas Hospital, Athens;
[5]1st Department of Propaedeutic Surgery, Hippokration Hospital, University of Athens, Greece

Background: Leptin is a potent direct angiogenic factor that stimulates endothelial cell migration and activation *in vitro*, and angiogenesis *in vivo*. In addition, leptin seems to play an important role in angiogenesis as it promotes the formation of new blood vessels. *Objective:* To determine the effect of local application of exogenous leptin on the survival of full thickness skin flaps in an experimental animal model. *Materials and Methods:* Ninety Sprague-Dawley rats were used. A full thickness dorsal flap (10 cm × 2 cm) with the pedicle located at the level of the iliac crest was designed. Animals were divided into ten groups of nine animals each. In the distal two thirds of the flap and by means of subdermal injection at 8 different locations, rats were injected with 100 ng/ml leptin, 250 ng/ml leptin, 500 ng/ml leptin, 1000 ng/ml leptin (groups A, B, C and D), 1 μg/ml VEGF (group E), or 1ml saline (control group), respectively. For each of the four leptin doses used, another animal group was injected with a combination of leptin/antileptin: 100 ng/ml leptin with 150 ng/ml antileptin, 250 ng/ml leptin with 375 ng/ml antileptin, 500 ng/ml leptin with 750 ng/ml antileptin or 1000 ng/ml leptin with 1500 ng/ml antileptin (groups A1, B1, C1 and D1, respectively), in order to study the inhibition of the leptin factor. Nine rats served as controls and were injected with 1 ml saline solution. Rats were sacrificed 3, 7 and 9 days postoperatively. After sacrifice of the animals, the skin was grossly arranged on its appearance, color and texture. Full thickness skin flaps were dissected for histological examination. A qualitative analysis of angiogenesis in the flap was conducted following a standard hematoxylin and eosin stain. The wound tissue samples from each experimental group underwent immunohistochemical evaluation of microvessel density by endothelial cell staining with mouse anti-rat CD34 monoclonal antibody. *Results:* Immunohistochemical staining revealed that more granulation tissue and improved angiogenesis were observed in group D (1000 ng/ml leptin) flaps compared to those in the VEGF, leptin/antileptin and saline groups. In addition, skin flap survival rate in group D (1000 ng/ml leptin) and group E (1 μg/ml VEGF) were significantly better than those of the other groups. The most impressive formation of new blood vessels was noted in the groups with the higher leptin doses. Surgical wounds in the control, as well as in the leptin/antileptin groups, did not demonstrate any new vessels. *Conclusion:* Exogenous administration of recombinant leptin increases early skin flap angiogenesis in an experimental animal model. Local application of leptin could efficiently improve survival of ischemic skin flaps.
Dr Liapakis has a scholarship from "The Hellenic Anticancer Institute".

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TAMOXIFEN. PHARMACOGENOMICS, DRUG INTERACTIONS, AND THERAPEUTIC DRUG MONITORING

Ernst A. Lien

Hormone Laboratory, Haukeland University Hospital, 5021 Bergen, Norway

Tamoxifen is regarded as a pro-drug as two of its metabolites, 4-hydroxytamoxifen and 4-hydroxy-*N*-demethyltamoxifen (endoxifen), have a higher affinity for the estrogen receptor than tamoxifen itself. 4-Hydroxy-*N*-demethyltamoxifen is considered the predominant active metabolite of tamoxifen because its pharmacological potency is equivalent to that of 4-hydroxytamoxifen and its concentration in blood and tissues is several fold that of 4-hydroxytamoxifen. Tamoxifen metabolism shows considerable inter-individual variation in normal dose as well as low-dose regimens.

The bioconversion of tamoxifen involves *N*-oxidation, *N*-demethylation and hydroxylation. The *N*-oxidation of tam is primarily catalyzed by a flavin-containing monooxygenase. Tamoxifen *N*-demethylation to *N*-demethyltamoxifen and N-dedimethyltamoxifen is catalyzed predominantly by the inducible cytochrome P450 (CYP) 3A4. CYP2D6 hydroxylates tamoxifen and *N*-demethyltamoxifen to the clinically potent metabolites 4-hydroxytamoxifen and 4-hydroxy-*N*-demethyltamoxifen. Furthermore, 4-hydroxy-*N*-demethyltamoxifen and 4-hydroxytamoxifen are conjugated and inactivated by the polymorphically distributed UDP-glucuronosyltransferase 2B15 and the sulfotransferase 1A1.

CYP2D6 activity is highly polymorphically distributed in the population due to inherited mutations in the *CYP2D6* gene. The poor metabolizer phenotype carries a combination of two non-functional variant alleles, which results in lack of CYP2D6 activity in the liver. In contrast, the ultrarapid metabolizer phenotype is associated with an inherited duplication of the *CYP2D6* gene that may increase enzymatic activity. Although CYP2D6 is the major contributor to the hydroxylation process of tamoxifen, *in vitro* studies using recombinant human CYPs have indicated an additional contribution by the enzymes CYP2B6, CYP2C9, CYP2C19 and CYP3A4. While the results vary, multiple studies from different groups have found an association between non-functional CYP2D6 alleles and poor outcome among individuals treated with tamoxifen.

The observed major interindividual variation in the serum levels of tamoxifen during steady state treatment has several causes. Breast cancer patients often use drugs that interact with CYP2D6 and the inducible CYP3A4. Furthermore, a low willingness of breast cancer patients to participate in clinical studies with tamoxifen has been observed. Moreover, the pharmacokinetics of tamoxifen varies by age.

The tissue levels of tamoxifen and its metabolites are related to their levels in serum. Accordingly, therapeutic effects as well as side-effects of the drug may be related to its serum levels. We therefore propose the inclusion of therapeutic drug monitoring in clinical tamoxifen studies.

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TUMORIGENICITY AND ANGIOGENESIS OF PANCREATIC CANCER ARE INHIBITED IN FAT1 (ω-3 DESATURASE) TRANSGENIC MICE

Kyoung-Sub Song[1], Eun-Jin Yun[1], Hae-Duck Park[3], Jong-Seok Kim[1], Jun-Young Heo[1], Yeon-Joo Jung[1], Chang Han[4], Tong Wu[4], Jing X. Kang[5], Jong-Il Park[1], Gi-Ryang Kweon[1], Wan-Hee Yoon[1], Byung-Doo Hwang[1,2] and Kyu Lim[1,2]

[1]Department of Biochemistry, College of Medicine; [2]Cancer Research Institute, Chungnam National University, Daejeon, Korea; [3]Dr. Park's Breast Clinic, Daejeon, Korea; [4]Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, PA15213, USA; [5]Department of Medicine, Massachusetts General Hospital and Harvard medical School, Boston, MA 02114, USA

ω3-Polyunsaturated fatty acids (ω3-PUFAs) including docosahexaenoic acid (DHA) are known to inhibit cell growth through apoptosis in various types of cancer cells. However, the mechanism of ω3-PUFA-induced cell death is still unclear. In this study, we have investigated inhibition of tumorigenicity and angiogenesis of ω3-PUFAs on pancreatic cancer in Fat1 transgenic mice (Fat1 transgenic mice express a *Caenorhabditis elegans* ω3 desaturase, converting ω6- to ω3-PUFAs endogenously).

Treatment of cells with two ω3-PUFAs, DHA and eicosapentaenoic acid (EPA), significantly and dose-dependently induced inhibition of cell growth and increased sub-G1 population in SW1990 and PANC-1 cells. The effect of ω3-PUFAs was due to induction of apoptosis, given that DHA induced cleavage of PARP and caspase-3 activity; in contrast, arachidonic acid (AA) had no effect. Moreover, DHA treatment reduced the level of β-catenin in SW1990 and PANC-1 cells. DHA also induced the association of β-catenin/Axin/GSK-3β complex, which serves as a parallel mechanism for β-catenin degradation. Increased nuclear staining for β-catenin was observed in tumor tissues prepared from human pancreatic cancer patients when compared with the non-tumor pancreatic tissues. In immunocytochemical stain of β-catenin, the cytosolic and nuclear β-catenin expression was reduced in the DHA-treated SW1990 cells as compared to control cells. The TCF/LEF reporter activity, a β-catenin-controlled downstream signal, was also reduced by DHA treatment. DHA also inhibited Cox-2 reporter activity in a dose-dependent manner. When mouse pancreatic cancer PANC-02 cells were implanted into Fat1 mice and wild-type mice, respectively, tumor size and volume of Fat1 transgenic mice was dramatically reduced as compared with wild mice. In immunohistochemical analysis of tumors of Fat1 mice, apoptosis was markedly increased and angiogenesis was significantly reduced as compared with wild-type mice tumors. Neoangiogenesis in Matrigel was dramatically inhibited in Fat1 mice compared to wild mice.

These findings suggest that ω3-PUFAs induce apoptotic cell death through the Cox-2 and β-catenin/wnt signaling pathway in human pancreatic cancer cells. Furthermore, They also provide evidence that ω3-PUFAs may inhibit tumorigenicity and angiogenesis of pancreatic cancer in the Fat1 transgenic mice model. Therefore, utilization of ω3-PUFAs may represent a potential effective therapy for the chemoprevention and treatment of human pancreatic cancer. This work was supported by Yihang Scholarship Foundation of Chungnam National University Medical School, and Dr. Park's Breast Clinic, Korea.

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OMEGA-3 POLYUNSATURATED FATTY ACIDS SUPPRESS CELL INVASION AND ANGIOGENESIS BY INHIBITION OF MMPs/COX-2/ VEGF THROUGH NF-κB AND AP-1 SIGNAL IN BREAST CANCER

Eun-Jin Yun[1], Kyoung-Sub Song[1], Hae-Duck Park[3], Jong-Seok Kim[1], Jun-Young Heo[1], Yeon-Joo Jung[1], Chang Han[4], Tong Wu[4], Jing X. Kang[5], Jong-Il Park[1], Gi-Ryang Kweon[1], Wan-Hee Yoon[1], Byung-Doo Hwang[1,2] and Kyu Lim[1,2]

[1]Department of Biochemistry, College of Medicine; [2]Cancer Research Institute, Chungnam National University, Daejeon, Korea; [3]Dr. Park's Breast Clinic, Daejeon, Korea; [4]Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, PA15213, USA; [5]Department of Medicine, Massachusetts General Hospital and Harvard medical School, Boston, MA 02114, USA

Omega-3 polyunsaturated fatty acids (ω3-PUFAs) are known to inhibit cancer cells proliferation in animal models and cell

lines. In contrast, ω6-PUFAs promote the growth of cancer cells. However, the molecular mechanisms remain to be elucidated. This study was designed to investigate action mechanisms of ω3-PUFAs, especially docosahexaenoic acid (DHA), on inhibition of invasion and angiogenesis in breast cancer.

Treatment of human breast cells (MDA-MB-231, T47) with two ω 3-PUFAs, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), for 12-48 hours resulted in a dose- and time-dependent inhibition of cell growth; in contrast, arachidonic acid (AA), a 6-PUFA, had no significant effect. The ω3-PUFAs effect are due to the induction of apoptosis, given that DHA induced the cleaved form of PARP, and caspase-3. DHA treatment caused a decline of β-catenin protein. Accordingly, DHA treatment also reduced the β-catenin-mediated T-cell factor/lymphoid enhancer factor reporter activity, and inhibited the expression of cyclin D1, a β-catenin-controlled downstream gene implicated in breast carcinogenesis. DHA suppressed motility and invasion of MDA-MB-231 cells in a dose-dependent manner; in contrast, AA had no effect. In gelatin-embedded zymography, activities of MMP-2 and MMP-9 were decreased by DHA treatment and the levels of their mRNA were also reduced in dose-dependent manner. The levels of VEGF protein and its promoter activity were inhibited by DHA treatment. AA- and PGE2-induced VEGF promoter activity was also inhibited by DHA pretreatment. The level of NF-κB protein and binding activities of NF-κB and AP-1 decreased by DHA treatment. DHA inhibited NF-κB and Cox-2 reporter activities and PGE2-dependent increases of NF-κB and MMP-2 promoter activities were also inhibited by DHA pretreatment. When mouse breast cancer EO771 cells were implanted into Fat1 mice (Fat1 transgenic mice express a *Caenorhabditis elegans* ω3 desaturase, converting ω6- to ω3-PUFAs endigenously), tumor size and volume of Fat1 transgenic mice were dramatically reduced compared with wild-type mice. In immunohistochemical analysis of tumors of Fat1 mice, apoptosis was markedly increased; in contast, angiogenesis significantly was reduced compared with wild-type mouse tumor. Binding activites of NF-κB and AP-1 were decreased in Fat1 mouse tumors. These findings suggest that ω3-PUFAs may inhibit tumor invasion and angiogenesis by reducing MMP activities and VEGF level *via* reduction of NF-κB and AP-1 in breast cancer. Thus, utilization of ω3-PUFAs may represent an effective and safe therapeutic approach for the chemoprevention and treatment of human breast cancer.

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ULTRASOUND-INDUCED HYPERTHERMIA AFFECTS THE CELLULAR UPTAKE OF P-GLYCOPROTEIN-RECOGNIZED SUBSTRATES IN MULTIDRUG-RESISITANT CELLS

Cheong-Weon Cho[1], Kyu Lim[2] and Sang-Chul Shin[3]

[1]College of Pharmacy, Chungnam National University, Daejeon, 305-764, South Korea;
[2]Department of Biochemistry, College of Medicine, Chungnam National University, Daejeon, 301-747, Korea;
[3]College of Pharmacy, Chonnam National University, Gwangju, 500-757, South Korea

Ultrasound-induced hyperthermia (USHT: 0.4 watts (W)/cm^2 at 41˚C) could increase the cellular uptake of p-glycoprotein (P-gp) substrates in bovine brain microvessel endothelial cells (BBMECs). This study was conducted to elucidate the mechanism of USHT on the cellular accumulation of P-gp-recognized substrates in the multi-drug resistant (MDR) cells since P-gp plays a major role in limiting drug permeability in MDR cells. When the concentration of P-gp inhibitor, verapamil or PSC 833, increased from 1 μM to 200 μM, verapamil and PSC 833 revealed a dose-dependent increase in R123 accumulation in MDR cells. To determine if USHT mediated its effect on the cellular accumulation of hydrophobic molecules by affecting membrane permeability, we compared the cellular accumulation of a non-P-gp hydrophobic substrate ([^{14}C]-antipyrine) with that associated with a P-gp substrate (R123). Antipyrine was chosen as a hydrophobic permeability marker, because this molecule is not considered a substrate of P-gp or of any known transporter proteins in MDR cells. Cellular accumulation of both 4 μM of R123 (log partition coefficient 0.53) and 0.5 μM of [^{14}C]-antipyrine (log partition coefficient 0.4) was increased immediately after USHT treatment: the accumulation of [^{14}C]-antipyrine reached a level that was 2.5-fold higher right after UHST treatment, whereas R123 accumulated only 33% over the control (No USHT) right after USHT. The enhanced permeability was reversible and size-dependent as USHT produced a much larger effect on cellular accumulation of [^{14}C]-antipyrine (MW 188) than that of R123 (MW 380.8). It also took less time after USHT treatment for R123 accumulation in USHT-treated cells to return to that of the untreated cells (20 min for R123 *vs.* >60 min for antipyrine). The results suggest that USHT does not modulate P-gp activity, but rather it increases cellular accumulation of R123 by altering membrane permeability in a manner that was selective and reversible. The present results point to the potential use of USHT to increase cellular uptake of P-gp-recognized substrates, especially anticancer agents, into cancer cells.

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INDUCTION OF APOPTOSIS BY CANTHARIDIN IN COLO 205 HUMAN COLORECTAL CARCINOMA CELLS

Chin-Chung Lin[1], Jai-Sing Yang[2], Jing-Gung Chung[3]

[1]Fong-Yuan Hospital, Fong Yuan City 420, Taiwan;
[2]Department of Pharmacology, China Medical University, Taichung, Taiwan;
[3]School of Biological Science and Technology, China Medical University, Taichung, Taiwan, ROC

Cantharidin, a natural toxin, is the active substance of mylabris and has antitumor effects in man. The present study was designed to investigate whether or not cantharidin exerts cytotoxic activity against colorectal cancer cells by inducing apoptosis and to examine the possible mechanism of the phenomenon. Inhibition of proliferation of cantharidin on COLO 205 colorectal cancer cells was determined by the trypan blue dye exclusion test *in vitro*. Apoptosis of cantharidin-treated cells was determined by morphological analysis and quantities by flow cytometry after staining with propidium iodide. Cell cycle and the cell surface expression of the CD95/CD95 ligand were evaluated by flow cytometry. Caspase activities were also analyzed. *Results:* Treatment with cantharidin of COLO 205 cells not only inhibited cell proliferation, but also induced apoptosis. Cantharidin induced apoptosis mainly in two phases: rapid apoptosis and delayed apoptosis in G_2/M arrested cells. Treatment with cantharidin in COLO 205 cells resulted in an up-regulation of the CD95 receptor and CD95L on the cell surface. Cantharidin-treated cells exhibited the activation of caspase-8 and caspase-3. The pretreatment of zVAD-FMK (a broad range caspase inhibitor) and/or IETD-FMK (a caspase-8 inhibitor) showed apparent inhibition of the apoptosis-inducing effect in COLO 205 cells. Our results suggest that cantharidin triggers apoptosis in colorectal cancer cells *via* the activation of the CD95 receptor/ligand system, and that this agent may be useful for developing new therapeutic regimens for the treatment of colorectal carcinoma.

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RUTIN INHIBITS HL-60 LEUKEMIA CELLS *IN VIVO*

Jing-Pin Lin[1], Guang-Wei Chen[2] and Jing-Gung Chung[3]

[1]School of Chinese Medicine, [3]Department of Biological Science and Technology, China Medical University, Tiachung, Taiwan;
[2]Traditional Chinese Medical Department, Chung-Ho Memorial Hospital, Kaohsiung Medical University, Kaohsiung, ROC

It was reported that rutin, a flavonoid present in onions, apples, tea and red wine, can induced apoptosis in cancer cells through the induction of apoptosis, however, the effects of rutin on human leukemia HL-60 cells is unclear. Therefore, the purpose of this study was to investigate the effect of rutin on HL-60 cells *in vitro* as well as *in vivo* systems. Nude mice bearing HL-60 leukemia tumors were treated once per 3 days with *i.p.* administration of 30 and or 50 mM rutin in 0.9% NaCl solution for 4 weeks. Tumor volume and survival time were recorded. TUNEL assay and CD34 vessel staining were conducted in tumor tissue. Antiangiogenesis *in vivo* was determined by sponge assay. Antiproliferative and apoptosis-inducing activities of rutin *in vitro* were examined on HL-60 cells. The results of the administration of rutin were significant inhibition (60%-80% maximum inhibition relative to controls) of the growth of HL-60 tumor xenografts, and prolonged survival of the treated mice. Complete tumor regression occurred in rutin-treated nude mice. The antitumor responses were associated with marked increases in tumor apoptosis and reductions in tumor size and weight. In conclusion, our data indicate that rutin may provide an effective approach to inhibit human leukemia HL-60 cancer both *in vitro* and *in vivo*.

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ORPHAN NUCLEAR RECEPTOR, NURR-77 IS A POSSIBLE TARGET GENE OF BUTYLIDENEPHTHALIDE CHEMOTHERAPY IN GLIOBLASTOMA MULTIFORM BRAIN TUMOR

Po-Cheng Lin[1], Yi-Lin Chen[2], Tzyy-Wen Chiou[1] and Horng-Jyh Harn[3]

[1]Graduate Institute of Biotechnology, National Dong Hwa University, Hualien, Taiwan;
[2]Graduate Institute of Biotechnology, National Ilan University, Ilan, Taiwan;
[3]Pathology Department, China Medical University, Taichung, Taiwan, ROC

The natural compound *n*-butylidenephthalide (BP), which is isolated from the chloroform extract of *Angelica sinensis*, has been investigated for its antitumoral effects on glioblastoma multiform (GBM) brain tumors both *in vitro* and *in vivo*. In order to determine the mechanism of BP-induced growth arrest and apoptosis, we examined BP-induced changes in gene expression by microarray screening using human GBM brain tumor cells. This analysis identified several BP-inducible genes, including the nuclear receptors *NOR-1, Nurr1,* and *Nur77*. Among these genes, *Nur77* is particularly interesting because it plays an important role in the apoptotic processes in various tumor cell lines. BP was able to increase *Nur77* mRNA and protein expression in a time-dependent manner. After BP treatment in GBM 8401 cells, Nur77 translocated from the nucleus to the cytoplasm, cytochrome *c* was released

from the mitochondria, and caspase-3 became activated. Furthermore, using *Nur77* promoter-luciferase assay, BP-increased Nur77 was AP1-related. Inhibition of BP-induced Nur77 expression by *Nur77* short interfering RNA (siRNA) blocked BP-induced apoptosis in GBM 8401 cells, suggesting that the induction of *Nur77* negatively affected GBM 8401 cell survival. In summary, our results suggest that up-regulation of Nur77 may explain the antitumoral activity of BP in brain tumor cells.

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DNA DAMAGE AND ENDOPLASMIC RETICULUM STRESS MEDIATED CURCUMIN-INDUCED CELL CYCLE ARREST AND APOPTOSIS IN HUMAN LUNG CARCINOMA A-549 CELLS THROUGH THE ACTIVATION CASPASES CASCADE AND MITOCHONDRIAL-DEPENDENT PATHWAY

Song-Shei Lin[1], Hsuan-Pang Huang[2], W. Gibson Wood[3] and Jing-Gung Chung[2]

[1]Department of Radiological Technology, Central Taiwan University of Science and Technology, No 11, Buzih Lane, Buzih District, Taichung 40605, Taiwan, ROC;
[3]Department of Pharmacology, University of Minnesota, School of Medicine, Geriatric Research, Education and Clinical Center, VA Medical Center, Minneapolis, Minnesota, USA;
[2]Departments of Biological Science and Technology, China Medical University, No 91, Hsueh-Shih Road, Taichung 404, Taiwan, ROC

Curcumin, a major component of the Curcuma species, is known to have antioxidant, anti-inflammatory properties and induce apoptosis of cancer cells, however, the precise molecular mechanisms of apoptosis *in vitro* are unclear. In this study, we showed that curcumin, a plant product containing the phenolic phytochemical, caused DNA damage and endoplasmic reticulum (ER) stress and mitochondrial-dependent-induced apoptosis through the activation of caspase-3 at a treatment concentration of 30 μM in human lung cancer A-549 cells. In contrast, treatment with 5-10 μM of curcumin did not induce significant apoptosis, but rather induced G_2/M phase arrest in A-549 cells. Flow cytometric analysis indicated that curcumin directly increased intracellular oxidative stress based on the cell permeable dye, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), acting as an indicator of reactive oxygen species (ROS) generation. GADD153 and GRP78 were increased by curcumin which was indicative of ER stress. Curcumin increased Ca^{2+} levels and the mitochondrial membrane potential ($\Delta\Psi m$) decreased in A-549 cells. Overall, our results demonstrated that curcumin treatment causes cell death by activating pathways inducing G2/M phase arrest and apoptosis.

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THE ROLE OF BRIT1/MCPH1 IN CHROMATIN REMODELING, DNA DAMAGE RESPONSE, AND CANCER

Guang Peng and Shiaw-Yih Lin

Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX 77054, USA

We previously identified *BRIT1/MCPH1* as a novel tumor suppressor gene and an early regulator in response to DNA damage through its regulation of ATM/ATR signaling as well as the expression of BRCA1 and Chk1. In addition to its role in DNA damage signaling, our recent endeavor indicated that BRIT1/MCPH1 was required for both homologous recombination DNA repair and nonhomologous end joining repair. Interestingly, using a proteomic approach, we identified a previously unknown function of BRIT1/MCPH1 as a novel regulator of ATP-dependent chromatin remodeling complex SWI/SNF in DNA repair. Our data indicate that BRIT1/MCPH1 interacts with SWI/SNF and provides a means by which SWI/SNF can be specifically recruited to and maintained at DNA lesions, facilitating DNA repair. We establish that loss of BRIT1/MCPH1 causes impaired chromatin relaxation in response to DNA damage owing to reduced association of SWI/SNF with chromatin. This then explains the observed decreased recruitment of repair proteins to DNA lesions and reduced efficiency of repair in BRIT1/MCPH1-deficient cells. Our findings, therefore, identify BRIT1/MCPH1 as a key molecule that directly links chromatin remodeling with DNA damage response in the control of DNA repair, and its dysfunction contributes to genomic instability and cancer.

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BREAST CANCER AND GROWTH HORMONE RECEPTOR EXPRESSION IN YOUNG ARAB WOMEN

David T. Lincoln[1], Fatma Al-Yatama[2], Fawziah M.A. Mohammed[2] and Anwar Al-Banaw[2]

[1]Entity Systems, Independent Research Foundation, Chapel Hill, QLD, Australia;
[2]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, Kuwait

Growth hormone (GH) exerts its regulatory functions in the mammary gland by acting on specific receptors, which trigger a cascade of biochemical signals and dictate gene expression. However, GH induced neoplastic changes have been reported in mice and human mammary gland tissue. In this investigation, the expression of receptors for growth hormone (GH-R) was undertaken in 122 cases of breast cancer in young

Arab women. In 24% of the patients, the tumour was very large, involving half the breast. The majority of cases were infiltrating ductal carcinomas (66%), of which 59% were moderately differentiated G2 or were poorly differentiated G3. The remaining cases included benign breast tumours, intraductal and lobular carcinomas *in situ* and invasive lobular and medullary carcinomas. Using anti GH-R monoclonal antibody (MAb 263) on routinely formalin-fixed, paraffin wax-embedded tissue sections, the cellular and nuclear expression of human GH-R was demonstrated by the streptavidin-biotin horseradish peroxidase complex (ABC) technique. The MAb used in this study was obtained by hybridoma technology from Balb/c mice immunised with purified rat and rabbit liver GH-receptor. The results show distinct receptor immunoreactivity in carcinoma cells. GH-R expression was primarily in the membrane-cytoplasm of cancer cells, but nuclei were also positive (16-30%) for GH-R. The proportion of membrane-cytoplasmic and nuclear expression in intraductal carcinomas was greater than 31% and 5-15% respectively. Strongest staining intensity was present in the large proliferating tumour cells. All cases of infiltrating ductal carcinomas were GH-R-positive with a high (>31%) positive nuclear component in this tumour etiology. The majority of ductal carcinomas were GH-R-positive, the proportion of immunoreactivity ranging from 5% to greater than 31%, while in lobular carcinomas, positive immunostaining was from 16% to greater than 31%, with nuclear expression being less than 5%. In signet ring carcinomas, cellular and nuclear GH-R expression was in excess of 31% and 16-30% respectively. All metastatic lymph node cases were positive for GH-R. Expression in normal breast tissue was generally low or absent. In conclusion, this study demonstrates the expression of receptors for growth hormone, an important mammotrophic hormone, in breast cancer tissue. It is proposed that growth hormone enhances cellular proliferation by acting on sensitive ductal stem cells, rendering them targets for the proliferative effects of IGF-I, resulting in propagation of genetic errors. Growth hormone also facilitates cell proliferation through paracrine and/or autocrine mechanism. It also suggests caution in the use of recombinant GH in the treatment of short stature prepubescent girls. This applies to those without evidence of classic GH deficiency, particularly in the presence of a family history of breast cancer.

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EXPRESSION OF GROWTH HORMONE RECEPTORS IN HUMAN LYMPHOMAS

David T. Lincoln[1] and Anwar Al-Banaw[2]

[1]Entity Systems, Independent Research Foundation, Chapel Hill, QLD., Australia;
[2]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, Kuwait

The classification of non-Hodgkin's lymphoma is controversial in histopathology. The Kiel and the Lukes-Collins classifications are widely used for the diagnosis of B-cell and T-cell lymphomas. In contrast, peripheral T-cell lymphomas are less well characterized and their identification is often difficult as the result of a multiplicity of immunological, histological and clinical characteristics. There is a growing appreciation of the role of the immune response in preventing the emergence and progression of cutaneous lymphomas, particularly with respect to local imbalances in immune regulations, creating an environment for tumourigenesis.

Growth hormone (GH) has been amply implicated as having a modulatory role in immune functions. Hyophysectomised rats and dwarf mice have suppressed immune responses, which can be restored by GH. Human GH stimulates T-cell proliferation, possibly via induction of insulin-like growth factor-I (IGF-I) synthesis, and the generation of cytotoxic T-cells is enhanced by GH. IGF-I synthesis in Epstein-Barr virus-transformed B cell lines is increased in response to GH. Furthermore, GH has been shown to stimulate B-cell immunoglobulin synthesis and proliferation. Clinically, GH treatment results in a number of changes in lymphocyte subpopulations and stimulates the proliferation as well as the transformation of normal and leukaemic lymphocytes *in vitro* and significantly increases the expression of c-myc proto-oncogene. GH induced neoplastic changes have been reported in lymphosarcoma of the lung and in plasma cell tumours.

To address the site/mode of action through which GH exerts these effects in a variety of human lymphomas, in this study we have applied a well-characterized monoclonal antibody to the GH-receptor (GHR), directed against the hormone-binding site of the receptor. Tumours investigated consisted of malignant non-Hodgkin's lymphoma (n-14), large cell malignant non-Hodgkin's lymphoma (n=11), non-Hodgkin's lymphoma-plasmacytoma (n=1), mycosis fungoides (n=11), low grade skin lymphoma (n=7), lymphoma/Kaposi's sarcoma (n=4), malignant lymphoid neoplasm (n=2), polymorphic lymphoid cells (n=1), cutaneous T-cell lymphoma (n=4) and adult T-cell leukaemia/lymphoma (n=7). Tumours were categorized for different lymphocyte subpopulations by immunomorphological diagnosis.

The results show strong GHR expression in the great majority of $CD20^+$ B-lymphocyte lymphomas, whereas $CD2^+$ lymphomas, including T-cell lymphomas, exhibited considerably lower levels of expression. The different levels of GHR expression observed on B- *vs*. T-lymphocytes were previously confirmed on peripheral lymphocytes by analysis of the *GHR* mRNA transcript. Cutaneous lymphomas, identified as highly malignant Ki-lymphoma of large anaplastic cells, expressed intense GHR immunoreactivity. Possibly the tumours presented here are a separate entity within the group of peripheral T-cell lymphomas, closely

related to Mycosis fungoides d'emblee. GHR staining was localized in a homogenous pattern and most of the neoplastic cells were positive. At times, nuclei were GHR positive in the tumour cells.

In conclusion, this study confirms ubiquitous expression of GHR on peripheral lymphomas and reveals discretely higher GHR levels in cutaneous B-cell compared to T-cell lymphomas. It strongly supports the idea that GH is directly involved in the control of the development and/or activation of specific cell populations of the immune system and that B- and T-cells may exhibit differential sensitivity to GH. The effects of GH may be particularly important at ages when the lymphoid compartment is still, to a certain extent, immature. On this basis, it would be of interest to reevaluate the biological effect of GH on the immune system of children with growth retardation undergoing GH treatment, and the possible carcinogenic and/or tumour growth stimulating effects of this treatment. A possible relationship is postulated between exogenous GH therapy and an increased risk of lymphoproliferative malignancy, including leukaemia growth-stimulating effects.

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HISTOPATHOLOGICAL ASPECTS AND RISK FACTORS OF BREAST CANCER IN YOUNG ARAB WOMEN

David T. Lincoln[1], Fatma Al-Yatama[2], Fawziah M.A. Mohammed[2] and Anwar Al-Banaw[2]

[1]Entity Systems, Independent Research Foundation, Chapel Hill QLD 4069, Australia;
[2]Department of Medical Sciences, Faculty of Allied Health Sciences, Kuwait University, Kuwait

Breast cancer is characterized by a long history and marked heterogeneity in growth rates and clinical manifestations. In Western women, peaks between the ages of 60-65 years, with only 14% occurring below the age of 40 years. In contrast, in Arab women the mean age is 48 years and the peak occurrence is 45-50 years. Only 14% of breast cancer occurs above the age of 60 years. However, a significant proportion of breast cancers occur below the age of 30 years and the incidence is increasing. The combination of obesity, hypertension and diabetes mellitus seems to be in close correlation with onset of the disease. Furthermore, the tumour size and the number of positive lymph nodes reflect the status of the cancer.

This study evaluates the expression of different prognostic tumour markers, including HER-2/neu oncoprotein, growth hormone receptor (GH-R), p53 oncoprotein, oestrogen receptor (OR), progesterone receptor (PR), cathepsin D, microvascular density (MVD), tumour cell proliferation (PCNA, Ki-67), thioredoxin (TRX) and thioredoxin reductase (TR). A total of 122 cases of breast cancer in young (<30 years old) Arab women were investigated and correlated with histopathological grade and lymph node status.

In 24% of the patients the tumour was very large, involving half of the breast; 80% presented without distant metastasis. According to the TNM staging system, most cases were T2, with an average tumour size of 3.5 cm (1.5-6.5).The larger the size of breast tumour, the more likely was the involvement of axillary lymph nodes. Stage I represented 14% of the patients, 44% were in stage II, 24% in stage III and 13% in stage IV. The average number of lymph nodes dissected was 18 (6-43). Results from this investigation show strong expression of HER-2/neu was present in 65.6% of breast carcinomas. Immunoreactivity was concentrated in the cell membranes of tumour cells. Among the 14 cases of infiltrating ductal carcinomas, 10 were strongly HER-2/neu positive and associated with lymph node involvement, while only 4 cases were moderately positive. The intensely positive cases of the intraductal carcinomas were all lymph node positive, indicating a relationship between HER-2/neu overexpression and lymph node involvement. The strongly positive cases of lobular, infiltrating lobular and signet ring carcinomas correlate with lymph node involvement.

Growth hormone receptor (GH-R) was expressed in all cases of infiltrating ductal carcinomas with a high (>31%) positive nuclear component in this tumour etiology. The majority of ductal carcinomas were positive with proportion of membrane-cytoplasmic and nuclear expression ranging from 5% to greater than 31%. In signet ring carcinomas, the proportion of GH-R immunoreactivity was in excess of 31% in the membrane-cytoplasmic component and 16-30% of the nuclei of tumour cells. The proportion of membrane-cytoplasmic and nuclear expression in the intraductal carcinomas was greater than 31% and 5-15% respectively. With 57.4%, distinct nuclear p53 staining was high, indicating alterations in *p53* tumour suppressor gene of breast carcinomas in young patients, and while 63.7% of these tumours were aneuploid, 93.6% of these were negative for OR and 64.8% of the p53-positive tumours were lymph node negative. The vast majority of p53-positive carcinomas were Grade III (76.6%), 21.3 % were Grade II and only 2.1 % Grade I, but neither tumour grade (p=0.066) nor tumour size (p=0.53) showed a correlation with p53 expression. A total of 60.9% of the p53-positive breast carcinomas had positive lymph node involvement. A significant negative correlation between OR and PR content (p=0.006) and p53 was observed.

Of the breast carcinomas from patients of young age investigated, 39% stained positive for cathepsin D, which was present in all grades of malignancy, including low Grade I tumours. Generally, only a small number of the carcinoma cells displayed distinct Cathepsin D immunoreactivity, which was confined to the cytoplasm only. Immunoreactivity of the MVD marker CD-31 was present in the vascular endothelial

cells of the larger blood vessels and capillaries. All other components of the breast carcinoma tissue, including tumour cells, stromal cells, adipocytes and nerve cells, were CD-31-negative. Ki-67 cell cycle immunoreactivity was present to varying degree in all breast carcinomas investigated and was mostly confined to the nuclei of the tumour cells. Cells in mitosis at times also displayed weak staining in their cytoplasm. The number of cells displaying Ki-67-positive nuclei varied between 9% and 89% of all malignant cells in a given tumour specimen. PCNA immunoreactivity was present in all breast carcinomas investigated (2% to 100%). The range of positive cells in the majority of carcinomas was between 20 to 35% of the tumour cells. Immunostaining of Ki-67 and PCNA specifically demonstrated proliferating tumour cells and correlated with tumour grade. The histochemical analysis of both TRX and TR shows they were overexpressed in the cytoplasm and nuclei in the majority of mammary carcinomas. TRX was found in 100% of the signet ring carcinomas, 100% of the mucin-producing carcinomas, 80% of the infiltrating ductal carcinomas, 70% of the ductal carcinomas, 60% of the intraductal carcinomas and 40% of the lobular carcinomas. In contrast, only 20% of the fibrocystic breast diseases investigated was TRX positive. Thioredoxin reductase (TR), the enzyme involved in the reduction of TRX, was also present in large amounts in the breast tumours, indicating that a large amount of its substrate (TRX) is also present. TR was present in 100% of the signet ring carcinomas and in the mucin producing carcinomas, 80% of the infiltrating ductal carcinomas, 60% of both the ductal and intraductal carcinomas and 30% of all lobular carcinomas investigated.

Patients <30 years of age have a worse prognosis than older patients. Only 6.5% of the tumours were classified as Grade 1, while 25.4% were of Grade II and 68.1% were Grade III. Furthermore, 14.6% of all carcinomas were <2 cm in size, 53.3% were between 2.1 and 5.0 cm and 28% were larger than 5 cm in size. In 42.8% of all cases, there was no lymph node involvement, 15.9% had one to three and 41.4% had four or more lymph node metastases, while 41.8% of all carcinomas were diploid and 58.4% were aneuploid. Grading correlated significantly with ploidy (p=0.008). DNA aneuploidy was more common in high-grade tumours. All of Grade I tumours, 47.4% of Grade II and 61.6% of Grade III tumours were aneuploid.

In conclusion, there are highly significant trends for the prevalence of poor prognostic features such as Grade 3 histology, extensive intraductal component and vascular and lymphatic invasion. The application of new biochemical markers for the diagnosis of breast cancer is valuable in therapeutic assessment and in clinical prognosis and has influenced the treatment selection. Expression of Ki-67, PCNA, p53 and HER-2/neu correlated significantly with each other and with the histological grade. Many of the carcinomas showed a high Ki-67 and PCNA proliferative activity, indicative of poor prognosis. A positive correlation was also demonstrated between tumour grade, nuclear proliferation and expression of GH-R, HER-2/neu, TRX and TR. A direct role for GH in breast cancer cell and in vascular epithelial cells proliferation suggests caution in the use of recombinant GH in the treatment of short stature prepubertal girls. This applies to those without evidence of classic growth hormone deficiency, particularly in the presence of a family history of breast cancer. Secreted TRX can regulate the redox state of specific cell surface receptors and of the extracellular environment. Consequently, TRX may exert a redox control over cellular mechanisms that are involved with cell attachment, invasion and metastasis and that the extracellular TRX system contributes to the invasive capacity of breast cancer cells. Given the association of the thioredoxin system with the process of tumourigenesis and malignancy reported here, the targeted inhibition of the TRX system is suggested as a novel approach for the treatment of aggressive breast cancer phenotypes. Indeed, specific chemical inhibitors of TRX and TR are known to inhibit cancer cell growth and exhibit antitumour activity *in vivo*. MVD was prognostically significant in univariate analysis of disease-free status and overall survival, as were stage of the disease, tumour size, nodal status and histological grade. High-grade carcinomas contained greater MVD than low-grade carcinomas. On the other hand, a negative correlation existed with OR and PR status and a weak correlation was found between HER-2/neu, Ki-67, PCNA, GH-R and p53 on one hand and Cathepsin D on the other. The association of both oestrogen and progesterone receptor-negative hormone status and positive p53 expression points to a greater tumour aggressiveness. This may reflect changes in steroid metabolism and usually excludes tamoxifen therapy. Most early recurrences and aggressive growth were present in high grade tumours with size of more than 3 cm. Development of recurrent metastatic disease was 22% of patients at intervals ranging from 1 month up to 12 years.

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HOW DO WE DEVELOP EFFECTIVE CYTOTOXIC DRUGS FOR SOLID TUMORS?

Stig Linder

Cancercenter Karolinska, R8:00, Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden

Clinically used anticancer agents such cisplatin induce both tumor cell death and terminal growth arrest. They do so by complex mechanisms, involving both nuclear and cytoplasmic targets (Mandic *et al*: J Biol Chem 278, 9100, 2003; Berndtsson *et al*: Int J Cancer *120*: 175, 2007). Despite considerable potency (and toxicity), these drugs do generally not cure metastatic disease.

Most currently used anticancer agents were develop using tumor cell lines grown in monolayer culture. Our group has

used a cell-based high-throughput apoptosis assay to identify agents that induce apoptosis in p53-defective cells (Erdal *et al*: PNAS *102*: 192, 2005), that induce the lysosomal cell death pathway and agents that induce apoptosis in cells overexpressing AKT.

A problem in this field is that monolayer cultures of tumor cells poorly reflect the properties of *in vivo* tumors. Tumor cells *in vivo* grow in a three dimensional matrix, and are often hypoxic and poorly nutritioned. Many currently used anticancer drugs are ineffective on 3-D multicellular spheroids. We have sucessfully adapted spheroids for drug screening using the M30-Apoptosense assay (Herrmann *et al*: J Biomol Screening *13*: 1, 2008). We have identified natural products and other compounds that induce efficient apoptosis of multicellular spheroids, and have characterized the mode of action of these drugs. We hope that agents that induce apoptosis of spheroids will also be effective *in vivo*.

Clinical testing represents an important part of the drug development process. Our laborarory has together with a biotech company, developed a method for determination of an apoptosis product released from dead tumor cells (Kramer *et al*: Cancer Res *64*: 1751, 2004). This method has proven useful for determination of clinical response after treatment with effective drugs (Hägg Olofsson *et al*: Clin Cancer Res *13*: 3198, 2007).

In conclusion, it is hope that improved cell-based screening systems together with effective determination of drug response in clinical trials will result in the development of more effective drugs against solid tumors.

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LRIG AND CERVICAL CANCER

Annika K. Lindström

Ventrum Clinic, Skovägen 2, 79021 Bjursås, Sweden

Cancer results from a multistep process in which acquisition of self sufficiency in growth signals is an important step. Growth factor receptors are frequently amplified and/or inappropriately activated in carcinoma cells. Three human leukokine-rich repeats and immunoglobulin like domains (LRIG) genes and proteins, named LRIG1-3, that may act on as promoters and/or suppressors of tumor growth have been characterized recently. We analyzed the LRIG gene family members in human cervical cancer. The relationship between LRIG protein expression and clinical parameters, ten other tumor markers, smoking, endogenous sex hormones and survival rate were investigated. LRIG1 appears to be a significant prognosis predictor in early-stage squamous cervical cancer. Diminished expression in advanced stages and the inverse correlation to serum progesterone and smoking indicates that LRIG1 is a tumor suppressor in cervical cancer. Expression of LRIG2 was strongly associated to poor prognosis, particularly in early stage squamous cell cervical cancer. There were correlations between LRIG2 expression and expression of the tumor suppressor LRIG1, the oncoprotein c-myc, the cell-adhesion molecules E-cadherin and CD44, and the immunological marker CD4. A combination of high LRIG2 expression and absence of LRIG1 expression identified women with a very poor prognosis The results of this study suggest that LRIG2 act as a tumor promoter in cervical cancer. According to our preliminary results LRIG 3 does not have any clinical importance in cervical cancer.

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TUMOUR MARKERS AND PROGNOSIS OF RECURRENCE AND SURVIVAL AFTER LIVER SURGERY FOR COLORECTAL LIVER METASTASES – A DYNAMIC STUDY

V. Liška[1], L. Holubec Jr.[2,3], V. Třeška[1], T. Skalický[1], A. Sutnar[1], S. Kormunda[3], J. Fínek[2] and O. Topolčan[3]

Departments of [1]Surgery, [2]Oncology, [3]Central Isotopic Laboratory, University Hospital Pilsen, Charles University Prague, Prague, Czech Republic

Aim: Statistical analysis of dynamic of tumour markers and comparison of tumour markers and their dynamics in patients without or with liver surgery. *Methods:* Log-rank test and Wilcoxon test were used for statistical evaluation. Survival rate and disease free interval (DFI) were computed by Kaplan-Meier method. We analysed serum levels of tumor markers conventionally used in clinical praxis (CA19-9, CEA, CA72-4) and markers informing us of the proliferative activity of malignancy (TK, TPA, TPS). The authors studied 51 patients who underwent explorative laparotomy or laparoscopy without any surgical therapy for colorectal liver metastases (CLM) and 82 patients who underwent radical liver surgery for CLM between September 1999 and June 2005. *Results:* The statistical analysis proved conventional tumour markers as prognostic factors of survival rate for patients after explorative laparotomy ($p<0.05$). The survival rate did not depend on the proliferative activity of malignancy. The statistical analysis of the dynamics of tumour markers after liver surgery (speed and power of recurrence) proved the dynamics of CA19-9 and CEA to be excellent prognostic factors of early recurence of CLM, contrary to proliferative tumor markers. *Conclusion:* The results of this study suggest the importance of tumor markers for the prediction of a short survival rate or DFI. It seems to be very helpful for planning of palliative oncological therapy for patients with liver malignancies who could not be treated by surgical therapy. Patients with a high tendency to recurrence of CLM after liver surgery should be followed-up more thorougly to increase the possibility of reoperation.

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USE OF CYTOKINES OF INFLAMMATORY RESPONSE IN PROCESS OF LIVER REGENERATION IN PORCINE MODEL OF PARTIAL PORTAL VEIN LIGATION

V. Liska[1], V. Treska[1], H. Mirka[2], J. Kobr[3], R. Sykora[4], T. Skalicky[1], A. Sutnar[1], J. Bruha[1], O. Fiala[1], O. Vycital[1], A. Chlumska[5], L. Holubec[6], M. Matejovic[4], J. Racek[7], L. Trefil[7] and O. Topolcan[6]

Departments of [1]Surgery, [2]Radiology, [3]Pediatrics and [7]Biochemistry, [4]First Department of Medicine, [5]Institute of Pathology and [6]Central Radioicotope Laboratory, Charles University Prague, Medical School and Teaching Hospital Plzen, Prague, Czech Republic

Background: Portal vein ligation (PVL) could multiply the future liver remnant volume. TNF-α and IL-6 are cytokines of inflammatory response and are connected with initial phase of liver regeneration. *Aim:* The aim of this study was to accelerate regeneration of liver parenchyma after PVL. The experimental porcine model was developed to be as compatible as possible with PVE in human medicine. *Methods:* The animals were divided into three groups: 10 piglets in a TNF-α group, 9 piglets in an IL-6 group and 8 piglets in a control group. After ligation of portal branches of caudate and right lateral and right medial liver lobes, recombinant porcine TNF-α, IL-6 or physiological solution (control group) were applied into non-occluded portal vein branches. The blood samples were collected from central vein catheter (1) before operation, (2) after ligation of the last portal branch, (3) during application of cytokine, (4) 2 hours after application of cytokine, (5) 1st postoperative day (p.d.), (6) 3rd p.d., (7) 7th p.d., (8) 10th p.d. and (9) 14th p.d. The following biochemical and immunoanalytical parameters were assessed: bilirubin, urea, creatinine, alkaline phosphatase, gammaglutamyltransferase, cholinesterase, aspartate aminotransferase, alanine aminotransferase, albumin, C-reactive protein, studied cytokines (TNF-α and IL-6) and growth factors (HGF, TGF-β1, TGF-α, IGF). After termination of the experiment, tissue samples for histological examination were acquired. Ultrasonographic examinations were undertaken immediately after operation and on 3rd, 7th, 10th and 14th p.d. day to evaluate the compensatory hypertrophy. *Results:* The acceleration of growth of hypertrophic liver lobes measured by ultrasonography was maximal between the 3rd and 7th postoperative day in the TNF-α group and on the 7th postoperative day in the IL-6 group in comparison with the control group ($p<0.05$), nevertheless this stimulating effect was lost at the end of experiment. Important differences in the biochemical, immunoanalytical or histological parameters studied were not proven. *Conclusion:* The presented study describes a newly developed experimental model of portal vein ligation in a large animal. The achieved acceleration of growth of hypertrophic liver lobes after application of TNF-α or IL-6 confirmed their key roles in priming of regenerating liver parenchyma after portal vein ligation.

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NEW DEVELOPMENTS IN EPIGENETIC THERAPY OF CANCER

Delong Liu and Shundong Cang

Division of Hematology/Oncology, New York Medical College, Valhalla, NY 10595, USA

Aberrant DNA methylation and histone acetylation play very important roles in carcinogenesis. Gene silencing through epigenetic mechanisms are now well established as a hallmark of cancer cells. Histone deacetylase inhibitors (HDACIs) and hypomethylating agents (HMAs) can reverse these processes through epigenetic therapies. To date, one HDACI (vorinostat) and two HMAs (azacitidine and decitabine) have been approved for clinical treatment of malignancies (Table). More than 13 new agents are in clinical trials for therapy of a variety of cancer types (Table). This report will summarize new findings from clinical trials and provide updates on the new epigenetic agents.

	Number of trials		
Agent	Phase I	Phase II	Phase III
Azacytidine	1	2	3
Azacytidine +	6	2	
Decytabine	4	5	2
Decytabine +	4	2	
Vorinostat	6	8	
Vorinostat +	8	1	
CI-994	3	1	
FK228	3	2	
FK228 +	1		
ITF2357		1	
LBH589	1	1	
LBH589+	1		
PCI-24781	1		
Phenylbutyrate	4	1	
MGCD103	4	2	
MGCD103+	1		
MS-275	2	1	
PXD101	2	1	
PXD101+	1		
SNDX-275+	1		
Valproic acid	1		
Valproic acid +	2		

+ indicates that the agent was in combination with another agent in the clinical trials.

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A NOVEL FLUOROMETRIC ASSAY OF HUMAN 3'-PHOSPHOADENOSINE 5'-PHOSPHOSULFATE SYNTHETASE

Tzu-An Liu[1] and Yuh-Shyong Yang[1,2]

[1]Department of Biological Science and Technology and Institute of Biochemical Engineering, National Chiao Tung University, Hsinchu, Taiwan;
[2]Instrument Technology Research Center and National Nano Device Laboratories, NARL, Hsinchu, Taiwan, ROC

Posttranslational modifications of proteins have been recognized as critical modulators of various physical activities in health and disease. Sulfation, catalyzed by sulfotransferases, is a key posttranslational modification modulates the extracellular cell-host and cell-matrix interactions, cell adhesion, as well as cell recognition and invasion, which are generally thought to remarkably influence carcinogenesis. 3'-phosphoadenosine 5'-phosphosulfate synthetase (PAPSS) catalyzes the biosynthesis of 3'-Phosphoadenosine 5'-phosphosulfate (PAPS), which serves as the universal sulfate donor compound for all sulfotransferase reactions. In an attempt to explore the enzymatic characteristics of PAPSS, we have developed and optimized a rapid, coupled fluorometric assay for the determination of PAPSS activity. This assay replaced end-point radioisotopic method and is the first continuous method. PAP-free phenol sulfotransferase proved to be ideal under the present situation because of its higher tolerance to substrate inhibition by PAP and MU, and exhibited the relative resistance to the inhibition by ATP, a substrate for PAPSS-catalyzed step. The selective phenol sulfotransferase was therefore utilized to catalyze the sulfate conjugation of an acceptor, MU, served as fluorometric indicator for determining PAPSS activity, by transferring the sulfuryl group from PAPS formed from ATP and inorganic sulfate by PAPSS. This rapid and continuous method can be used in the detailed pharmacogenetic and toxicological studies of PAPSS, and will allow for investigation of other pathological mechanisms for a better understating of the regulation between sulfation and tumor procession.

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HMJ-38 INTERACTS WITH MICROTUBULE POLYMERIZATION, PROMOTE G_2/M ARREST AND INDUCES APOPTOSIS IN HUMAN TONGUE CANCER CAL27 CELL LINE

Chi-Cheng Lu[1], Jai-Sing Yang[2], Sheng-Chu Kuo[3], Ming-Ching Kao[4], Mann-Jen Hour[5] and Jing-Gung Chung[4]

[1]Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan;
[2]Department of Pharmacology, China Medical University, Taichung, Taiwan;
[3]Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung, Taiwan;
[4]School of Biological Science and Technology, China Medical University, Taichung, Taiwan;
[5]School of Pharmacy, China Medical University, Taichung, Taiwan, ROC

We reported that HMJ-38 was the most potent 2-phenyl-4-quinozolinone derivative in inhibiting tubulin polymerization and it showed significant cytotoxicity against human leukemia HL-60 cell. In this study, HMJ-38 was examined for its growth inhibition effect on human tongue cancer CAL27 cells. After 48 hours treatment of HMJ-38 on CAL27 cells, the MTT method was applied to determine the proliferation rate and a dose- and time-dependent decrease in cell proliferation was observed. The IC_{50} of HMJ-38 for CAL27 cells was 2.5 μM. Cell cycle analysis and microscopic examination of CAL27 cells showed that HMJ-38 induced significant G_2/M arrest and apoptosis. We found that HMJ-38 up-regulated the protein levels of p-CDK1 (Thr161), Chk1, Chk2 and p21, and increased cyclin B and CDK1 kinase activity. HMJ-38-treated CAL27 cells presented typical characteristics of apoptosis as further evidenced by DAPI/TUNEL staining and increase of internucleosomal DNA cleavage. In addition, apparent release of cytosolic cytochrome *c*, pro-caspase-9, Apaf-1 and AIF, down-regulation of Bcl-2, Bcl-xL, xIAP, up-regulation of phospho-Bcl-2, Bax and Bad, and cleavage of pro-caspase-3 and pro-caspase-9 were detected by Western blotting. Caspase activity assay and sub-G1 nuclei analysis of HMJ-38-treated CAL27 in the absence or the presence of caspases inhibitors indicated that the HMJ-38-induced apoptosis was mainly mediated by the activation of caspase-9 and caspase-3. Our results suggest a mechanism of cytotoxic action of HMJ-38 and indicate that HMJ-38 may be a promising chemotherapy agent against human tongue cancer.

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GALECTIN-3: A USEFUL TUMOR MARKER IN THYROID AND COLORECTAL CANCER

Marie Ludviková[1,3], Luboš Holubec Jr.[2], Ivana Kholová[4], Josef Reischig[1] and Ondřej Topolčan[2]

[1]Institute of Biology, Medical Faculty, Charles University, Karlovarská 48, 301 66 Plzeň;
[2]Department of Oncology, Medical Faculty, Charles University, Plzen;
[3]Department of Pathology, 1st Medical Faculty, Charles University, Prague, Czech Republic;
[4]A.I.Virtanen Institute for Molecular Sciences, University of Kuopio, Finland

This review aims to summarize diagnostic and prognostic difficulties in thyroid and colorectal cancer from a pathologist's point of view, with emphasis on the role of galectin-3 in diagnosis and/or prognosis of these tumors. Results of our current galectin-3 studies will also be presented.

Galectin-3 (endogenous β-galactoside-binding protein) is a member of a group of widely distributed carbohydrate-binding proteins that have been implicated in a spectrum of physiological and pathobiological processes including malignant transformation. Galectin-3 was proven to be expressed in various cancer cells, mainly of thyroid and colorectal cancer. Its expression in these tumors has been intensively studied with respect to the diagnostic and prognostic role of this tumor marker.

Thyroid tumors of follicular cell origin represent a diagnostically and prognostically a difficult group of neoplasms. In particular differentiation between follicular adenoma and carcinoma, and between follicular tumor and follicular variant of papillary carcinoma await additional methods. Galectin-3 was produced in both benign and malignant thyroid tumors of follicular cell origin, but galectin-3 was significantly up-regulated in papillary carcinomas only in comparison with the other tumors ($p<0.0001$).

The main task at the time of a pathologist's diagnosis is the of assessment of the metastatic potential of colorectal cancer using several tumor markers. Liver metastatic lesions as well as primary tumors showed up-regulated mutually correlating levels of galectin-3.

Galectin-3 seems to be a promising diagnostic marker in malignant thyroid tumors of follicular cell origin, as well as a prognostic marker predicting metastasis in colorectal carcinomas.

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CLINICAL IMPLICATIONS OF EPITHELIAL OVARIAN CANCER PATHOBIOLOGY: AN OVERVIEW

Marie Ludvíková

Institute of Biology, Medical Faculty, Charles University, Karlovarská 48, 301 66 Plzen, Czech Republic

Ovarian tumors are heterogeneous neoplasms from morphological, pathobiological, and clinical aspects. Epithelial tumors of the ovary comprise about 60% of all ovarian neoplasms and more then 90% of ovarian malignancies. Epithelial ovarian cancer is the most lethal neoplasm of the gynecological malignancies. The early stage of ovarian cancer is often asymptomatic and more than two-thirds of patients are diagnosed with advanced disease.

The clinical outcome reflects the histological type and pathobiology of ovarian cancer. A dualistic molecular model of epithelial ovarian cancer elucidating clinical course has been proposed. Moreover, cancer stem cell theory could provide a unifying model of ovarian cancer, reflecting histologic heterogeneity and metastatic potential, as well as resistance to chemotherapy. However, molecular pathogenesis and progression of this disease are yet been definitively understood.

Currently histological diagnosis of ovarian neoplasms is based on their features in hematoxylin-eosin-stained sections, which continue to be the fundamental substrate of surgical pathology. Immunohistochemistry has assumed the prominent role in diagnostic ovarian pathology. Diagnostic tissue tumor markers serve for discrimination between microscopically similar pathological ovarian lesions. Unlike breast and colorectal cancer, no useful prognostic and predictive tissue tumor markers have been identified yet in ovary. Several promising prognostic and predictive ovarian tumor tissue markers (*e.g.* proliferative and angiogenetic markers, p53 overexpression, p16 decreased expression) have been studied so far, but none has proven to be useful for clinical application.

The clinical course of advanced disease is therefore difficult to predict in an individual patient. Dissimilarity of clinical outcome in patients with ovarian carcinoma suggests that reliable prognostic and/or predictive (tissue and/or circulatory) biological markers would be of potential clinical value and new treatment options are warranted.

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MALIGNANCY-RELATED HYPERCALCEMIA. PATHOPHYSIOLOGY AND TREATMENT

Franco Lumachi[1] and Umbertoi Basso[2]

[1]Department of Surgical and Gastroenterological Sciences, University of Padua, School of Medicine, 35128 Padova; [2]Division of Medical Oncology, Istituto Oncologico Veneto (IOV) IRCCS, 35128 Padova, Italy

Background: Hypercalcemia is a frequent complication of malignancy. Malignancy-related hypercalcemia (MRH) may occur in 5% to 30% of patients with cancer during the course of their disease, but its incidence varies with type of tumor. Lung cancer (*i.e.* squamous cell carcinoma), breast cancer and myeloma have the higher incidence of MRH, accounting for more than 50%, while the disease occurs rarely in patients with colorectal and prostate cancers. Usually, the prognosis of cancer patients with MRH is poor, with a mean survival rate of 2-3 months. However, in patients with breast carcinoma and multiple myeloma the course of the disease is characterized by self-limited episodes of hypercalcemia, which may be treated. *Pathophysiology:* Different mechanisms are responsible for hypercalcemia, enclos-ing (1)

degradation of bone matrix in patients with osteolitic lesions (*i.e.* lung, renal and head and neck cancer), (2) abnormal conversion of vitamin D3 within lymphoma cells and (3) secretion of parathyroid hormone-related protein (PTHrP), which repre-sents the principal pathway leading to hypercalcemia in cancer patients. Most hyper-calcemic patients with bone metastases may have also increased levels of PTHrP and the serum PTH measurement represents the first step in the differential diagnosis from benign (*i.e.* primary hyperparathyroidism) *versus* malignant hypercalcemia. Unfortu-nately, signs and symptoms (nausea, weackness, polyuria, lethargy, depression) are aspecific, but low PTH levels together with high calcium levels in a cancer patient may suggest a malignancy-related hypercalcemic syndrome. *Medical Treatment:* Vigorous intravenous hydration along with loop diuretics (*i.e.* furosemide) administration, which enhance renal excretion of calcium, represents usually the first step of the medical treatment. Old agents such as mithramycin, eth-ylphosphorothoric acid, calcitonin and gallium nitrate have practically been aban-doned due to their limited activity and huge side-effects, especially for the kidney. *Bisphosphonates:* Several bisphosphonates have shown to decrease serum calcium levels by inhibiting PTH-dependent osteoclast activation. Both clodronate and etidro-nate proved to be of some efficacy in patients with parathyroid cancer when given intravenously, while the oral formulation is inactive. Subsequently, more potent agents (*i.e.* pamidronate and zoledronate) have become available, and currently they represent the drugs of choice. Zoledronate is the most potent drug of this class, al-though periodical monitoring of renal function is strongly recommended for some cases of acute renal insufficiency have been reported. *Calcimimetic Drugs:* Since the production of PTH is negatively controlled by ionised calcium, agents able to potentiate this homeostatic control might be effective in sup-pressing PTH levels and reducing serum hypercalcemia. The prototype of these "cal-cimimetic" agents is cinacalcet, a new drug which interacts with the membrane-spanning segments of the CaSR and enhances signal transduction, presumably by inducing conformational changes in the caSR receptor which reduce the thres-hold for activation by ionised calcium and suppress PTH secretion in the absence of a change in the level of extracellular calcium. *Experimental Therapy:* A new experimental approach to treat malignant hyper-calcemia involves the blockade of receptor activator of nuclear factor-kappa B ligand, usually abbreviated as RANKL. RANKL is a key element in the differentiation, func-tion, and survival of osteoclasts, which play an essential role in removing Ca^{++} from the bone in response to PTH stimulation. Denosumab (AMG 162) is a new, fully hu-man monoclonal antibody against RANKL, which has been developed to antagonize osteolysis and PTH-related hypercalcemia.

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GENETIC MODELS IN BREAST CANCER RESEARCH

Leif R. Lund, Kasper Almholt, Boye S. Nielsen and John Rømer

Finsen Laboratory, Rigshospitalet, Copenhagen Biocenter, Denmark

Growth and invasion of breast cancer require extracellular proteolysis in order to physically restructure the tissue microenvironment of the mammary gland. This pathological tissue remodeling process depends on a collaboration of epithelial and stromal cells. In fact, the majority of extracellular proteases are provided by stromal cells rather than cancer cells. This distinct expression pattern is seen in human breast cancers and also in transgenic mouse models of breast cancer. The similar expression patterns suggest that transgenic mouse models are ideally suited to study the role of extracellular proteases in cancer progression. Indeed, these studies demonstrate that proteases are involved in all stages of breast cancer progression from carcinogenesis to metastasis.

Particular matrix metalloproteinases (MMP) have several roles that influence cancer progression and dissemination. However, low molecular weight metalloproteinase inhibitors (MPI) have not yet been tested in transgenic/spontaneous metastasis models. We have tested Galardin/GM6001,a potent MPI that reacts with most MMPs, in the polyoma virus middle T oncogene mouse model (MMTV-PymT) transgenic breast cancer model. Galardin treatment significantly reduced primary tumor growth. Final tumor burden in Galardin-treated mice was 1.69 cm^3 compared with 3.29 cm^3 in placebo-treated mice (*t*-test, p=0.0014). We quantified the total lung metastasis volume in the same cohort of mice and found that the metastasis burden was reduced more than 100-fold, whereas primary tumor size was reduced only 2-fold. We also found that primary tumors from Galardin-treated mice exhibited a lower histo pathologic tumor grade, increased collagen deposition, and increased MMP-2 activity.

During progression of mammary carcinomas in the MMTV-PymT model, MMP13 mRNA was strongly upregulated concurrently with the transition to invasive and metastatic carcinomas. As in human tumors, MMP13 mRNA was found in myofibroblasts of invasive grade II and III carcinomas, but not in benign grade I and II mammary intraepithelial neoplasias. To determine if MMP13 plays a role in tumor progression, we crossed MMTV-PymT mice with MMP13 deficient mice. The absence of MMP13 did not influence tumor growth, vascularization, progression to more advanced tumor stages, or metastasis to the lungs, and the absence of MMP13 was not compensated for by expression of other MMPs or tissue inhibitor of metalloproteinases. However, an increased fraction of thin collagen fibrils was identified in

MMTV-PymT;MMP13(-/-) compared to MMTV-PymT;MMP13(+/+) tumors, showing that collagen metabolism was altered in the absence of MMP13. We conclude that the expression pattern of MMP13 mRNA in myofibroblasts of invasive carcinomas in the MMTV-PymT breast cancer model recapitulates the expression pattern observed in human breast cancer. Our results suggest that MMP13 is a marker of carcinoma-associated myofibroblasts of invasive carcinoma, even though it does not make a major contribution to tumor progression in the MMTV-PyMT breast cancer model.

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EVALUATION OF THE CYTOTOXIC AND GENOTOXIC EFFECTS OF ORTHODONTIC BONDING ADHESIVES UPON A HUMAN GINGIVAL PAPILLAE THROUGH IMMUNOHISTOCHEMICAL EXPRESSION OF P53, P63 AND P16

Francesca Angiero[1], Enrico Dessy[2], Giampietro Farronato[3], Elisa Rossi[2], Sarah Magistro[3], Davide Farronato[3], Rossella Seramondi[3] and Stefano Tetè[4]

[1]Università degli Studi Milano-Bicocca, Anatomia Patologica;
[2]Università degli Studi di Brescia, Anatomia Patologica;
[3]Università degli Studi di Milano, Clinica Odontoiatrica;
[4]Università degli Studi di Chieti, Italy

Purpose: Numerous *in vitro* studies have shown that the composite materials have cytotoxic and genotoxic effects on cells they come into contact with. These materials are commonly used for restorations in conservative dentistry, and in orthodontics to anchor brackets to the tooth enamel. The study determined expression of p53, p63 and p16, biomarkers that are useful for predicting the potential genotoxicity of the monomeric adhesives used to anchor orthodontic brackets. *Patients and Methods:* Histological examination and P53, p63 and p16 expression were determined immunohistochemically on the gengival papillae of 99 patients, of which 69 had been banded orthodontically for one year; 30 patients without orthodontic banding were used as controls; brackets were bonded to teeth with a filled flowable composite resin. The papillae were removed surgically from patients and controls and examined both to evaluate morphological alterations and using immunohistochemistry to examine biological alterations. Associations between protein expression patterns and development of clinicopathologic parameters during follow-up were examined. *Results:* No case showed morphological alterations visible by microscope. Of the 69 banded patients, 4 (5.80%) were positive to p53, 2 exhibited positivity for p63 expression in the basal and suprabasal layers and were classified as p63 positive (2.90%). P16 was positive in one patient (1.45%). Of the control cases, no subject was positive for any of the biomarkers (0.00%). *Conclusion:* The significance of this positivity, together with histology, and whether these may serve as biomarkers to predict the risk of developing oral lesion (dysplasia, oral cancer) is still an open question. From this study it appears that, although the details of the mechanisms leading to cell death, genotoxicity, and cell-cycle delay are not fully understood, resin monomers may alter the functions of the cells of the oral cavity.

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DETECTION OF MINIMAL RESIDUAL DISEASE OF ACUTE CHILDHOOD LEUKEMIA BY FOLLOWING *WT1* GENE EXPRESSION IN THE PERIPHERAL BLOOD

Edina Magyarosy and Erzsébet Rásó

Tumor Progression Department of the National Institute for Oncology, Budapest

Cytomorphology and Southern blot technique are not sensitive enough to detect minimal residual disease and to diagnose relapse, and the different DNA markers, which are detected by other PCR techniques are present only in 20-30% of childhood leukemias. Several authors are of the opinion that monitoring *WT* gene expression in the peripheral blood can be used to monitor the progression of the disease, to detect minimal residual disease and to diagnose relapse early. The *WT1* gene has two splice regions and four different isoforms, which may play different roles in the disrupted expression of all WT1 isoforms. *WT1* gene expression in the bone marrow and in the peripheral blood in leukemia patients is well known in the literature, but the expression of WT1 isoforms has not been investigated yet.

The aim of our study was to investigate if monitoring *WT1* gene expression in the peripheral blood was an appropriate method to monitor the progression of acute childhood leukemias. In the course of the study, the peripheral blood of 27 newly diagnosed, 17 previously diagnosed and 21 nonleukemic children was tested for *WT1* gene expression. Twenty consecutive pediatric patients were included in this study for the analysis of the peripheral blood samples and the initial diagnosis of ALL for WT1 isoforms expression.

In agreement with the literature, we found that all ALL cases except one expressed the *WT1* gene (23/24, 96%). The level of WT1 expression did not correlate to the ratio of the blast cells in the peripheral blood. The initial high rate of WT1 positivity (23/24) became low (~21%) at the end of the induction phase of the therapy (day 15: 7/24, day 33: 5/24), although remission occurred in all the patients under treatment except one.

Twenty patients were followed for 12 months following induction therapy and 16 of them were further monitored for the second year (14-21 month). Clinical relapse occurred in two cases during the first year where the WT1 expression at

the periphery was maintained for 11-15 months and both patients died. On the other hand, there was no *WT1* gene expression found in the peripheral blood of non-leukemic hematological diseases, except myelodysplasia.

We were the first to examine the expression of different WT1 isoforms in the peripheral blood of children and we observed that the WT1 pattern is constant. We found that the 5 patients in the low-risk group expressed the 17AA(+) WT1 isoform, but 2 patients had the 17AA(–) WT1 isoform. We found the presence of 17AA(–) WT1 isoform in the high-risk group. The expression rates of 17AA(+) or 17AA(–) were the same in the medium risk group. This point of view was intermediate between the low-risk and high-risk group; no overexpression of 17AA(–) or 17AA(+) were observed. In two ALL cases, the suspicion of mutation in the WT1-17AA region arose, because we did not find PCR products in the 17AA region, but there was WT1 positivity in the KTS region.

Our results suggest that WT1 assays alone are not sufficient, but combined with the detection of other, widely used DNA markers, are useful to detect minimal residual disease. Following *WT1* gene expression levels in initially WT1-positive children enables us to diagnose relapse early, before it would be detectable with other existing methods.

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PRO AND CONS FOR CA-125 MONITORING IN THE FOLLOW UP MANAGEMENT

Sven Mahner

University Medical Center Hamburg-Eppendorf, Department of Gynecology and Gynecologic Oncology, 20246 Hamburg, Germany

Despite radical surgical and chemotherapeutic treatment of ovarian cancer, the majority of patients develop recurrent disease within two to three years. Because of this high likelihood of disease progression, most women are closely monitored after completing treatment. In case of disease recurrence, serum concentration of the tumor marker CA-125 usually rises 3-4 months prior to the development of symptoms or clinical and radiological signs of relapse. Although a cure of patients with relapsed ovarian cancer is rarely possible, it is still unclear whether an early reinduction of therapy could yield extended survival.

Potential benefits of an early treatment of recurrent disease could be the possible delay of symptoms and improved survival. The toxic side-effects and decreased interval without treatment in an otherwise asymptomatic patient are potential disadvantages.

Depending on the duration of response to previous therapy and the length of the treatment free interval, patients should be counselled on the advantages and disadvantages of serial measurement of CA-125 during follow up.

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CRITICAL FUNCTIONS OF HUMAN BILIVERDIN REDUCTASE IN INSULIN/IGF-1 AND MAPK SIGNALING: POTENTIAL APPLICATIONS IN TREATMENT OF DIABETES AND CANCER

Mahin D. Maines

Department of Biochemistry and Biophysics, University of Rochester School of Medicine, Rochester, NY 14642, USA

Degradation of heme to bilirubin involves stepwise conversion to biliverdin (BV) by heme oxygenases-1&2 followed by reduction of BV to the anti-inflammatory/antioxidant bilirubin by biliverdin reductase (BVR). In addition to its reductase activity, recent studies have revealed a diverse and expansive spectrum of functions of the human BVR, that is unmatched by any other protein. The wide assortment of structural features of hBVR underlies its function in multiple capacities in the cell . In this presentation, the current understanding of functions of hBVR that are highlighted below will be discussed.

hBVR is a dual-specificity kinase (S/T/Y) upstream activator of insulin/IGF-1 and MAPK signaling pathways. The hBVR is also a bZip DNA/chromatin binding transcription factor, an activator and anchor protein for translocation of PKC enzymes and transcriptional factors within cell compartments, and a kinase-kinase for activation of PKCs. Activation of the MEK/ERK/Elk-signaling cascade is a mechanism for relaying mitogenic and stress stimuli for gene activation. MEK1 is the proximate kinase for activation of ERK1/2, and nuclear targeting of ERK1/2 is obligatory for Elk1 transcriptional activity. Formation of a ternary complex between hBVR/MEK/ERK is essential for activation of ERK by MEK, and for transport of activated ERK into the nucleus, wherein another ternary complex is formed between hBVR/ERK/Elk that results in Elk1 activation and induction of gene expression. hBVR, its variants and small fragments have demonstrated ability to modulate cell signaling and, hence, the wide range of functions that are regulated by protein kinases that include growth, differentiation, gene transcription and metabolism. hBVR is nearly as effective as IGF in activating MEK-1-ERK axis, while eight-residue peptides, one flanking P^{165} and another containing S^{230} block the activation of ERK by IGF and PKC-ζ, by TNF-α, respectively, suggesting their utility as effectors of cell cycle progression and cell differentiation. Furthermore, because seven-residue peptide, KYCCSRK, can specifically bind hematin, it offers a rational approach to design compounds, based on the ligand-binding property, for delivering heme or synthetic heme analogues to induce or inhibit heme-regulated gene expression. The hBVR substrate, BV, and its product, BR, display notable effects in the cell. BV is an inhibitor of kinase and NF-κB activities, while BR is a quencher of

oxygen free radicals. BV inhibits NF-κB, and hBVR reverses this inhibition. This finding has the likely potential of providing a foundation for therapeutic intervention in inflammatory diseases and cancer that may be attained by preventing reduction of BV. On the other hand, by increasing BVR levels the beneficial functions of NF-κB might be augmented. In addition, the cytoprotective of BR against oxidative stress and free radical-linked diseases, as well as its cytotoxicity, are now amply documented. *Conclusion:* Regulation of glucose uptake, induction of HO-1, and cytokine and Toll-like receptor signaling are potential target candidates for hBVR-based therapeutic strategies. Because hBVR blocks free radical-promoted apoptosis, regulation of its activity presents a novel drug development strategy to prolong cell survival. Given the finding that hBVR-based 7or 8 residue peptides can effectively modulate cell-signaling networks, they bear the promise of developing into powerful tools for inhibiting or potentiating transmission of extracellular stimuli that control gene expression and cellular functions and to combat diseases that are associated with disruption of normal kinase-mediated functions such as diabetes and cancer .As additional functions of hBVR in cell signaling are uncovered, the prospect of the utility of hBVR-derived structures in therapeutic settings becomes increasingly more realistic. Studies were supported by NIH grants ES-012187 and ES-004066.

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CURCUMIN SYNERGIZES WITH COLON CANCER THERAPEUTICS: A STRATEGY TO ELIMINATE CANCER STEM CELLS

Althea A. Elliott, Bhaumik B. Patel, Deep-Shikha Gupta, Vivek Sengupta and Adhip P. N. Majumdar

John D. Dingell VA Medical Center, Karmanos Cancer Center, Department of Medicine, Wayne State University School of Medicine, Detroit, MI, USA

Despite the use of surgical resection and aggressive chemotherapy, nearly 50% of patients with colorectal carcinoma develop recurrent disease, highlighting the need for improved therapies. 5-Fluorouracil (5-FU) or 5-FU plus oxaliplatin (FOLFOX) remains the backbone of colorectal cancer chemotherapeutics, but produces incomplete response, resulting in survival of cells (chemo-surviving cells) that often leads to cancer recurrence, which could partly be due to proliferation of colon cancer stem cells. It is becoming increasingly evident that a cancer treatment that fails to eliminate cancer stem cells may allow regrowth of the tumor. The current treatment strategy for recurrent cancer consists mainly of aggressive screening, which is in itself costly, potentially morbid, and controversial. There is also a cost of additional toxicities, some of which are even fatal. Therefore, validation of a non-toxic agent that could improve upon the current chemotherapeutic regimen would be highly desirable.

Curcumin (diferuloylmethane), the major active ingredient of turmeric (*Curcuma longa*), used in South Asian cuisine, with no discernable toxicity, inhibits the growth of transformed cells and colon carcinogenesis at the initiation, promotion and progression stages in carcinogen-induced rodent models. Curcumin has also been shown to prevent the development of adenomas in the intestinal tract of Min+/– mice, a model of human familial adenomatous polyposis. In a Phase I clinical trial, curcumin was found to be effective in inhibiting the growth of a variety of tumors. The present investigation was, therefore, undertaken to examine whether addition of curcumin to FOLFOX will be a superior therapeutic strategy for colon cancer chemo-surviving cells, in part by eliminating colon cancer stem cells.

Forty-eight hour treatment of colon cancer HCT-116 or HT-29 cells (which contain 30-40% colon cancer stem cells) with FOLFOX (25 μM 5-FU+0.625 μM oxaliplatin) resulted in 60-70% cell survival, accompanied by a marked activation of IGF-1R and minor to moderate increase in EGFR, HER-2, as well as AKT, COX-2 and Cyclin-D1. However, inclusion of curcumin in continued FOLFOX treatment for another 48 h greatly reduced survival of these cells, compared to those subjected or not subjected to continued FOLFOX treatment (control). These changes were accompanied by concomitant reduction in expression and activation of EGFR, HER-2 and IGF-1R, as well as expression of down-stream signaling effectors such as AKT, COX-2 and Cyclin-D1. Moreover, 48 h treatment of chemo-surviving colon cancer HCT-116 cells with the combination of curcumin and FOLFOX markedly reduced the cancer stem cell population, as evidenced by their failure to form colonies, and a marked reduction in CD166 expression.

In conclusion, our data suggest that inclusion of curcumin to conventional chemotherapeutic regimen could be one of the effective strategies to prevent the emergence of chemo-resistant colon cancer cells by markedly reducing/eliminating the cancer stem cells.

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FUNCTIONALISATION OF APTAMERS FOR THE DEVELOPMENT OF EFFECTIVE CANCER THERAPEUTICS

V. Makwana[1], H. Khan[1], N. Courtenay-Luck[2] and S. Missailidis[1]

[1]Department of Chemistry and Analytical Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA; [2]Antisoma Research Ltd, Welwyn Garden City, UK

Introduction: Aptamers, short oligonucleotide therapeutic, diagnostic and imaging agents, possess great affinity and

selectivity for their targets. However, they are limited in terms of application due to their rapid renal clearance and degradation from nucleases in the blood. Thus, it is often necessary to incorporate appropriate modifications for achieving their full potential. Aptamer labelling with appropriate fluorescent moieties or radioisotopes can confer diagnostic and imaging potential, respectively. Various chemical modifications, such as PEGylation, can improve their pharmacokinetic properties and retain aptamers longer in circulation, making them suitable for therapeutic applications. We now present a range of chemical modifications on our tumour-targeting aptamers that have allowed us to develop them as diagnostic or therapeutic agents. *Methods:* The functionalisation of aptamers entails a covalent reaction between the fluorescent label, chelator or polyethylene glycol (PEG) polymer modified with an activated functional group and the previously selected aptamers in an aqueous solution at optimal pH and temperature. It is, however, possible to loose affinity to the target following extensive modification to the aptamer backbone or coupling to various agents. Fluorescence Resonance Energy Transfer (FRET), flow cytometry and cell cytotoxicity assays were carried out, following chemical modification, to determine the modified affinity and efficacy of the purified aptamer-conjugate for their target and ensure that modified aptamers retain their therapeutic properties. *Results and Conclusion:* Polyethylene glycol (PEG), a polymer varying in size and comprised of polymerized ethylene oxide monomers, has become a popular agent for modifying physiochemical and pharmacokinetic characteristics of therapeutic biological macromolecules. Conjugating therapeutic biological molecules to PEG has many positive attributes for clinical utilisation. The overall increase in the molecular weight of the targeting molecule enhances its residential and circulatory time within the body, thereby reducing renal clearance, which can be improved from hours to days, hence improving therapeutic efficacy. Other advantages include lower immunogenicity, increased protection from enzyme degradation and reduced toxicity. Similarly, the coupling of appropriately chosen fluorescent moieties can offer valuable immunoassay development potential for the identification and quantification of the target biomarker in biological media.

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INTRAUTERINE GROWTH RESTRICTION: PERINATAL PARAMETERS ASSOCIATED WITH FUTURE DEVELOPMENT OF THE METABOLIC SYNDROME

Ariadne Malamitsi-Puchner and Despina Briana

Neonatal Division, 2nd Department of Obstetrics and Gynecology, University of Athens, 76, Vas. Sophias Ave, 11528 Athens, Greece

Intrauterine growth restriction (IUGR) is the failure of the fetus to achieve his/her intrinsic growth potential, due to anatomical and/or functional disorders and diseases in the feto-placental-maternal unit. IUGR results in significant perinatal and long-term complications, including development of insulin resistance/metabolic syndrome in adult life.

The thrifty phenotype hypothesis suggests that intrauterine malnutrition leads to adaptational changes of the endocrine/metabolic mechanisms (programming), in order to secure fetal survival. In this respect, in IUGR fetuses, blood flow redistribution takes place, resulting in reduced mass/impaired function of pancreatic β-cells. Moreover, IUGR infants present with permanent changes in adipose tissue metabolic and hormonal functions. Adipose tissue regulates whole body metabolism, by secreting various hormones, named adipocytokines. The latter play a major role in the mechanisms linking obesity to insulin resistance/metabolic syndrome and were recently implicated in intrauterine growth.

A number of recent studies from our group explored the implication of adipocytokines (leptin, adiponectin, visfatin, resistin and apelin -also expressed by the human placenta) in fetal growth, by investigating and comparing circulating concentrations of these hormones in IUGR and appropriate for gestational age (AGA) fetuses and neonates. The relationship of the circulating concentrations of the above adipocytokines with respective insulin ones was also studied.

Our results, particularly referring to the novel adipocytokines indicate that resistin and apelin concentrations did not differ between IUGR cases and AGA controls and did not correlate with respective insulin ones, possibly indicating lack of direct involvement in perinatal insulin resistance and adipogenesis. In contrast, visfatin concentrations were higher in IUGR neonates, possibly implying increased visceral fats stores, as well as predisposition to insulin resistance. In this respect, visfatin may serve as an early marker with prognostic value for later development of insulin resistance/ metabolic syndrome. However, circulating insulin concentrations were lower in IUGR cases, possibly indicating reduced β-cell mass and/or impaired β-cell function. Furthermore, concentration of visfatin and apelin are high in the fetus, possibly due to placental expression. On the other hand, the fetus *per se* may contribute to its circulating resistin concentrations, as the latter did not decline after birth.

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EPIGENETIC CHANGES IN WILMS' TUMOURS

Anthony R. Dallosso, Anne L. Hancock, Laxmi Chilukamarri, Sally Malik, Keith W. Brown and Karim Malik

Cancer and Leukaemia in Childhood (CLIC) Sargent Research Unit, Department of Cellular and Molecular Medicine, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK

In addition to genetic events, Wilms' tumour (WT) aetiology has been closely linked with epigenetic lesions. WTs display epigenetic gene silencing, involving aberrant hypermethylation of tumour suppressor gene 5'-CpG islands. Our laboratory has previously demonstrated Wilms' tumour specific hypomethylation occurring at the *WT1* antisense regulatory region (*WT1* ARR) associated with loss of imprinting and increased expression of a regulatory RNA, *WT1-AS* and *AWT1*.

We have extended our studies with a genome-wide methylation analysis of WT and foetal kidney samples using human promoter microarrays hybridized with methylated DNA purified by 5-methyl-cytosine immunoprecipitation. Putative targets for epigenetic deregulation in WTs were then validated by COBRA analysis and gene expression quantified using real-time PCR. We report identification of long range epigenetic silencing (LRES) in Wilms' tumour. WTs display extensive hypermethylation of a large cluster of paralogous genes in this region. Further, on a distinct genetic locus, we show activation of gene expression associated with hypomethylation of *GLIPR1*. Our data show that WTs exhibit a wide range of epigenetic abnormalities, including hypermethylation, hypomethylation and loss of imprinting.

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THREE-DIMENSIONAL CELL CULTURE OF HUMAN GLIOMA CELLS AND MORPHOLOGICAL DIFFERENCES

Yoshinobu Manome and Michiko Watanabe

Department of Molecular Cell Biology, Institute of DNA Medicine Research Center for Medical Sciences, Jikei University School of Medicine 6-8-27 Nishishinbashi, Minato-ku, Tokyo, Japan 105-8461

Introduction: In vivo glioma grows three-dimensionally and spreads continuously by infiltrating the surrounding tissues. In contrast, cultured glioma cells lose their three-dimensional conformation and cease their proliferation when they reach confluence. In addition, vital cellular functions that are present in tissues or organs might be overlooked with ordinary 'culture dish'-based cell cultures. From this viewpoint, we attempted to establish a three-dimensional culture that mimics the local environment within the human body. We applied the method to human glioma cells and investigated their features. *Materials and Methods:* Bio-adaptable and degradable gelatin was meshed and used as a scaffold. Three malignant glioma cell lines, T98G, KNS42, and U118MG, were chosen for the experiments. Dispersed cells (1×10^4 cells/100 μl of DMEM) were attached to the 5-mm cubes of the scaffold. Cells were then further cultivated for 5 to 10 days. Specimens were examined by SEM, TEM, stereoscopy, and light microscopy. *Results:* Morphological examination of the three-dimension culture revealed features that were barely detectable in conventional cell culture. Steric cell-to-cell connections were observed throughout the culture and the culture showed characteristics of *in vivo* glioma cells. When the three gliomas were compared, each cell line presented a different form. U118MG cells were unpiled and attached to the scaffold with numerous fiber formations, whereas KNS42 cells aggregated, adhering to each other, thereby leading to the formation of built balloon-like structures. T98G cells showed an intermediate appearance. *Discussion:* Glioma cells grew three-dimensionally in the current culture. There were differences between the two- and three-dimensional cultures. Cell lines used for the study were representatives of standard gliomas. Although these cells are frequently used for many experiments, their features were quite different. This became evident only after using our culture. Under such circumstances, we conclude that three-dimensional cultures might be useful for further investigation of gliomas.

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PARSTATIN: A NEW CRYPTIC ANTI-ANGIOGENIC PEPTIDE

Michael E. Maragoudakis and Nikos E. Tsopanoglou

Department of Pharmacology, Medical School, University of Patras, Patras, Greece

Thrombin, the serine protease best known for its pivotal role in haemostasis, has been proposed to play an important role in the initiation of angiogenesis by a mechanism mostly independent of its coagulant activity and more dependent on signaling via the protease-activated receptors (PARs). PARs consist a novel family of G protein-coupled receptors, which are activated by proteolytic cleavage of their N-terminal extracellular domain. PAR-1 is the first member of this family to be cloned in which proteolytic cleavage at the R_{41}/S_{42} bond by thrombin releases a 41 aminoacid peptide and unveils a tethered peptide ligand with the recognition sequence SFLLRN. Despite the wealth of information relating to the role of thrombin and PAR-1 in physiology and disease states, a potential biological role of cleaved peptide remains unknown. We evaluated the effect of the 41-aa cleaved peptide in human endothelial cells as well as in *in vivo* and *ex vivo* and *in vitro* angiogenesis models. We have designated this fragment of PAR-1 as "parstatin". Exposure of endothelial cells to parstatin resulted in a concentration-dependent inhibition of serum-mediated proliferation, as well as of bFGF- and VEGF-induced cell growth. Consistently, parstatin blocked the serum-, bFGF- and VEGF-triggered Erk1/2 activation. In contrast, no effect was observed in cells treated with EGF or heparin-binding EGF. Growth inhibition of endothelial cells by parstatin was further confirmed by flow-cytometric cell cycle analysis. In addition, the Annexin V/propidium iodide apoptosis assay provided strong evidence

that the inhibition of cell growth is likely associated with induction of apoptosis. Parstatin increased the percentage of endothelial cells in early and late apoptotic stages in a concentration-dependent manner. Parstatin also triggered the activation of caspase 3 and the induction of poly (ADP-ribose)polymerase cleavage to its signature 85-kDa fragment. In addition, the apoptotic effect of parstatin was mostly reversed in the presence of caspase inhibitor Z-VAD-FMK. Parstatin abrogated tube formation in *in vitro* Matrigel and fibrin angiogenesis model and suppressed both the basic angiogenesis and that stimulated by VEGF or bFGF in rat aortic rings and in chick chorioallontoic membrane model *in vivo*. We have also shown that parstatin acts as a cell-penetrating peptide, exerting its biological effects intracellularly. These results provide a plausible evidence for a negative role of PAR-1 cleaved peptide in angiogenic cascade and suggest parstatin as target for developing anti-angiogenic agents with potential therapeutic application in cancer and other angiogenesis-related diseases.

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CONSTITUENTS OF *CARPOBROTUS EDULIS* INHIBIT P-GLYCOPROTEIN OF HUMAN *MDR1* GENE TRANSFECTED MOUSE LYMPHOMA CELLS

A. Martins[1,2], A. Vasas[3], Zs. Schelz[4], M. Viveiros[1], J. Molnár[4], J. Hohmann[3], G. Spengler[1,2] and L. Amaral[1,2]

[1]Unit of Mycobacteriology, [2]UPMM, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (IHMT/UNL), Rua da Junqueira, 96 1349-008 Lisbon, Portugal; [3]Institute of Pharmacognosy, Faculty of Pharmacy, University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary; [4]Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, H-6720 Szeged, Dóm tér 10, Hungary

Multidrug resistance is a major health problem that affects the therapy of cancer (eukaryotic cells) and MDR infections caused by *Escherichia coli, Staphylococcus aureus, Mycobacterium tuberculosis, etc*. Our findings suggest a new approach for the therapy of MDR cancer and bacterial infections that involves the use of these compounds as adjuvants to conventional therapies and have the potential to increase the usage of existing antibiotics that have fallen by the wayside due to MDR causes.

The methanolic extract of *Carpobrotus edulis* reverses resistance of mouse lymphoma cells that carry the human *mdr1* gene to chemotherapeutic agents and increases the killing activity of *Staphylococcus aureus* infected macrophages (1, 2). The aim of the present work was the identification of the compound(s) responsible for the MDR reversal activity.

A bioassay guided purification protocol was performed, testing the extracts, fractions and pure compounds for their ability to inhibit the p-glycoprotein (the efflux pump responsible for the multidrug resistance of this cell line) of mouse lymphoma cells containing the human efflux pump gene *mdr1*. The assay was performed following extrusion or retention of rhodamine 123 inside the cancer cells, by flow cytometry.

The compounds were isolated from the chloroform soluble fraction of the methanolic extract by means of multistep chromatographic separations, including CC, VLC, CPC, PLC and HPLC. The structures were determined by NMR investigations as triterpens (β-amyrin and oleanolic acid) monogalactosyldiacylglycerol and phenolic compounds.

The efflux pump inhibitory activities of some of these compounds against MDR mouse lymphoma cell were evaluated.

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THE TENSIN FAMILY OF PUTATIVE METASTASIS SUPPRESSOR PROTEINS ARE DOWN-REGULATED IN HUMAN KIDNEY CANCER

Danuta Martuszewska[1], Börje Ljungberg[2], Martin Johansson[3], Cecilia Oslakovic[1], Björn Dahlbäck[1] and Sassan Hafizi[1]

[1]Lund University, Dept. of Laboratory Medicine, Section for Clinical Chemistry, University Hospital Malmö, SE-205 02, Malmö;
[2]Umeå University, Department of Surgical and Perioperative Sciences, SE-901 85, Umeå;
[3]Lund University, Dept. of Laboratory Medicine, Section for Pathology, University Hospital Malmö, SE-205 02, Malmö, Sweden

Background: Renal cell carcinoma (RCC) is the most common form of kidney cancer in adults. In RCC, transformation and uncontrolled growth of epithelial cells occurs as a result of multiple molecular changes, part of which can be due to loss of tumor suppressor proteins. The Tensin family of intracellular proteins (Tensin1, -2, -3 and -4), are thought to mediate signal transduction of cell motility and growth and act as important links between the extracellular matrix and the cytoskeleton. Dysregulation of Tensin proteins has previously been implicated in various human cancers. Thus we hypothesize that at least some of the Tensins are novel tumor suppressors that may be dysregulated in RCC. *Aims:* The aim of the current study was to evaluate the significance of all four

Tensin isoforms in a study of human kidney cancer subjects. *Methods:* Quantitative real-time reverse transcriptase PCR was used to analyse mRNA expression of Tensins1-4 in human kidney samples, normal (n=48) *vs*. RCC (n=223). We also screened various human cancer cell lines for Tensin mRNA as well as protein expression by Western blot. Moreover, we performed immunohistochemical analysis of Tensin3 in human kidney tumors and generated stable Tensin3 expressing cells for migration assay. *Results:* Tensin2 and Tensin3 expression were found to be low or largely absent at both mRNA and protein levels in a panel of diverse human cancer cell lines. Messenger RNA expression of all four Tensin genes was significantly lower in human kidney tumors as compared to normal kidney tissue. Correlation studies revealed expression of the Tensins to correlate positively amongst each other, whilst they correlated negatively with tumor grade, but not tumor size. Immunohistochemical analysis revealed Tensin3 to be present in the cytoplasm of tubular epithelium in normal human kidney sections, and 40% of patient kidney tumor sections analyzed in microarrays showed Tensin3 expression to be low or absent. Overexpression of Tensin3 in human kidney 293 cells resulted in a significant reduction in migration capacity *versus* mock-transfected cells. *Conclusion:* Our findings indicate that the expression of all Tensins is downregulated in human kidney tumors and that loss of Tensins may lead to uncontrolled cell migration during metastasis. Furthermore anticancer therapies may benefit from inducing up-regulation of Tensins as a favourable side-effect.

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REGULATION OF C/EBPα BY HYPOXIA AND ESTROGEN IN BREAST CANCER CELLS

Liliane Massaad-Massade

Liliane Massade, Institut Gustave-Roussy, UMR 8121 Pavillon de recherche 2, 94805 Villejuif CEDEX, France

Experimental and clinical studies showed that both estrogen (E_2) and hypoxia (H) were involved in tumour development and progression. First, we undertake a study to determine whether these factors could interact to modulate gene expression using a microarray approach. We screened the transcript levels of over 8,000 genes in the estrogen receptor (ERα) positive T-47D human breast cancer cell line maintained at 21% O_2 or at 1% O_2 with or without E_2 co-treatment. Treatment by E_2 or hypoxia alone altered the expression of 26 and 9 genes, respectively, whilst the expression of 31 genes was modulated by H-E_2 combination. Of the 31 genes modulated by the H-E_2 combination, 21 of them were found to be down-regulated. Microarray data was validated for 19 by quantitative real-time PCR and a good correlation noted (r^2=0.8). Five out of these 19 genes were assayed for protein expression by Western blot and a good correlation was found between mRNA and protein levels. Statistical analysis showed that the gene expression modulation by the combined H and E_2 treatment was additive in most cases. Interestingly, the transcription factor CCAAT/enhancer binding protein-α (C/EBPα), involved in control of cell differentiation and proliferation, was found to be down-regulated by hypoxia and E_2. Therefore, we examined the mechanism by which the down-regulation by hypoxia and by E_2 takes place. Using the specific RNA polymerase II inhibitor 5,6-dichlorobenzimidazole 1-β-D-ribofuranoside (DRB), the mRNA stability was analyzed under normoxia and hypoxia, with and without E_2 co-treatment. Hypoxia and the H-E_2 combination but not E_2 alone reduced the half-life of the C/EBPα mRNA by ~30%. *C/EBPα* gene promoter studies indicated that hypoxia but not E_2 repressed the transcription of the gene and identified a hypoxia response element (-522; -527 bp) which binds to HIF-1α as essential for down-regulation of *C/EBPα* transcription in hypoxia. Immunocytochemical analysis showed that C/EBPα was localized in the nucleus at 21% O_2 with or without E_2 co-treatment, but was mostly cytoplasmic under 1% O_2 in the presence or in the absence of E_2. Knockdown of HIF-1α by RNAi restored C/EBPα to normal levels under hypoxic conditions. Immunohistochemical studies of 10 tumour samples did not show any colocalization of C/EBPα and GLUT1 (used as a marker for hypoxia). Taken together, these results show that hypoxia downregulates C/EBPα expression in breast cancer cells by several mechanisms, including transcriptional and post-transcriptional effects. The down-regulation of C/EBPα in hypoxia is mediated by HIF-1 that by the H-E_2 combination is mainly due to hypoxic effect. This justifies further examination of C/EBPα as a possible therapeutic target in breast cancer with large hypoxic regions.

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ROLE OF 2B4 (CD244) AND CS1 (CD319) IN NATURAL KILLER CELL-MEDIATED KILLING OF CANCER CELLS

Porunelloor A. Mathew and Stephen O. Mathew

Department of Molecular Biology and Immunology and Institute for Cancer and Blood Disorders, University of North Texas Health Science Center, 3500 Camp Bowie blvd., Fort Worth, TX, 76107, USA

Natural killer (NK) cells are components of the innate immune system and form the first line of defense against various cancer and viral infections. NK cells have the ability to kill certain cancer cells and their function is regulated by a delicate balance between activating and inhibitory signals received through cell surface receptors. 2B4 (CD244) and CS1 (CD319, CRACC) are members of the signaling lymphocyte-activation molecule (SLAM) family of receptors expressed on

NK cells. Activation of NK cells through surface 2B4 or CS1 enhanced the killing of several tumor cells including K562 and DU145 prostate cancer cells. Studies using *2B4* gene knockout mice revealed an *in vivo* role for 2B4 in rejection of B16 melanoma cells. 51Cr-release cytotoxicity assays showed that human NK cell line NK92MI activated with the 2B4 and CS1 monoclonal antibodies (mAb) were more effective in killing DU145 prostate cancer cells than unstimulated NK cells. Understanding the expression and function of these receptors will shed more light on how these receptors stimulate NK cells to effectively target and kill cancer cells. This will allow us to make attempts towards developing better immunotherapy treatments for cancer.

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ANTI-CANCEROGENIC ACTIVITIES AND ESSENTIAL BIOCHEMICAL PROPERTIES OF PLANT BIFUNCTIONAL NUCLEASE TBN1

Jaroslav Matoušek[1], Tomáš Podzimek[1,2], Pavla Poučková[3], Lidmila Orctová[1], Josef Souček[4], Jiří Škvor[5], Petra Lipovová[2] and Josef Matoušek[6]

[1]Biology Centre of the ASCR v.v.i., Institute of Plant Molecular Biology, Academy of Sciences of the Czech Republic, 370 05 České Budějovice;
[2]Department of Biochemistry and Microbiology, Institute of Chemical Technology Prague, 166 28 Prague;
[3]Institute of Biophysics and Information, Medical Faculty of the Charles University, 128 00 Prague;
[4]Institute of Hematology and Blood Transfusion, 128 00 Prague;
[5]Laboratory of Genetics, department of Anthropology, Faculty of Science of the Charles University, 128 20 Prague;
[6]Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, 277 21 Liběchov, Czech Republic

Recombinant tomato bifunctional nuclease 1 (TBN1) (E.C.3.1.30.x) that was cloned previously (1) was produced *in planta* by leaf agroinfiltration biotechnology, purified and analyzed for its antitumour and biological effects. Anticancerogenic and biological activities of TBN1 were analyzed in comparison with other plant nucleases and bovine ribonuclease (BS-RNase).

TBN1 displays cytotoxicity for human melanoma, prostatic carcinoma and neuroblastoma, growing in athymic mice. *In vitro* antiproliferative effect tested on ML-2 cell line was, however, negative. This enzyme in comparison with mung bean nuclease (PhA) (3) and (BS-RNase) was *in vivo* a little less effective on the melanoma tumours, however, more effective on melanoma in comparison to black pine pollen nuclease (PN) (4). The most antitumorous and antiproliferative effects were observed on prostatic carcinoma after intravenous application of TBN1 conjugated to polyethylene glycol (PEG) for its stabilization, suggesting selective action of TBN1 on cancer cells *in vivo*. It was found that TBN1 exhibits lower side-effects, *e.g.* aspermatogenesis of ICR mice, relative to other nucleases studied. As the most important fact, it was found that immunosuppressivity caused by TBN1 was almost zero in comparison to the high MCL immunosuppressivity characteristic.

As detected by immunofluorescence microscopy and by biochemical methods, TBN1 penetrates tumor cells within several hours after its application. The following biochemical properties that could be essential for the specific nuclease action on tumors are characteristic for purified TBN1. Mature nuclease TBN1 is a glycoprotein ~36 kDa. It has a pI of 5.42 and a pH optimum of 5.9 for double-stranded (ds) DNA and 6.3 for single-stranded (ss)RNA and DNA. It cleaves substrates ssRNA:dsDNA:ssDNA at the ratio 1:1.4:1.6. TBN1 exhibits 3'nucleotidase activity 0.54±0.02 μmol Pi/min/μg of protein at pH 6.0. The nuclease has capability to destroy human 28S, 18S, 7S and 5.8S RNA *in vitro,* as well as dsRNA producing oligo and mononucleotides. The enzyme shows maximum activity around 60˚C suggesting its high thermo- and structural stability. Hence, TBN1 can act on various cellular RNA species, genomic DNA, as well as on RNA induced during posttranscriptional gene silencing. Moreover, 3'nucleotidase activity of TBN1 can dephosphorylate some nucleoprotein receptor(s) or other cellular components.

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CULTIVATION OF PRIMARY MAMMARY EPITHELIAL CELLS FROM BREAST CANCER PATIENTS, BRCA1/2 CARRIERS, AND HEALTHY CONTROLS: USEFUL MODEL FOR BREAST CANCER RESEARCH

Eva Matouskova[1,2], Eva Bursikova[1,2], Jan Sevcik[1] and Zdenek Kleibl[1]

[1]Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University Prague, Prague 2, 121 08;
[2]Prague Burn Centre, Third Faculty of Medicine, Charles University Prague, Prague 10, 100 00, Czech Republic

In vitro cultured primary mammary epithelial cells (MECs) from oncological patients and healthy women represent one of the best models for studies of malignant transformation. We

have developed and optimized a feeder layer technique for cultivation of human MECs. More than 200 primary MEC cultures were established from normal and malignant tissue of the mammary gland. Among them, 26 cell populations from the mammary gland of *BRCA1* or *BRCA2* mutation carriers were prepared. Spontaneous immortalization occurred in two cell populations. The first one, EM-G3 cell line, was derived from primary breast carcinoma tissue and exerts characteristics of mammary progenitor cells. Recently, a second cell line from MECs has been established. Primary cells and cell lines were characterized by proliferative activity, morphology, dynamic activity (time-lapse cinemicrography), imunocytochemistry, cytogenetics and molecular biology. The MECs were used for development of the "Non-destructive Test of Cellular Activity" that can be used for individual tumors' chemosensitivity testing and for screening of new potentially antitumor drugs. The clonal cell line EM-G3 was able to partially differentiate *in vivo* as well as *in vitro*. It represents a unique spontaneously immortalized clonal cell line evidently derived from premalignant breast cancer progenitors. The study of differences in biological behavior between BRCA1 mutation-carrying MACs and *BRCA1*-wt MACs *in vitro* allows us to trace effects of *BRCA1* haploinsufficiency in early stages of mammary cancerogenesis in *BRCA1* mutation carriers.

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TELOMERE MAINTENANCE MECHANISMS IN MESENCHYMAL TUMORS

Toshihiro Matsuo[1], Takashi Sugita[1], Shoji Shimose[1], Tadahiko Kubo[1], Yuji Yasunaga[2] and Mitsuo Ochi[1]

Departments of [1]Orthopaedic Surgery and [2]Artificial Joints and Biomaterials, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

Background and Aim: Telomere maintenance is regarded as an important mechanism in evading senescence in tumor cells, and two types of telomere maintenance mechanisms have been described in human tumors: telomerase activation and alternative lengthening of telomeres (ALT). Approximately 85% of carcinomas engage a mechanism to maintain stable telomere length by telomerase activity. ALT is characterized by a heterogeneous pattern of telomere length, usually ranging from very short to abnormally long, and a substantial proportion of some types of sarcomas have been reported to have elongated telomeres consistent with ALT in the absence of telomerase activity. Therefore, sarcomas are distinct from carcinomas in that a substantial portion of them use the ALT mechanism to maintain their telomeres. We review the role of telomere maintenance mechanisms in bone and soft tissue tumors, including our recent results of an analysis of the clinical significance of telomere factors. *Recent findings:* The prevalence of ALT varied greatly among different soft tissue sarcoma subtypes and the proportion of ALT also differed among previous reports. In addition, the frequency of telomerase activity expression in mesenchymal tumors differed among previous reports. The telomere maintenance mechanism has recently emerged as a prognostic factor for sarcomas, although some of the data are controversial. *Our findings:* In giant cell tumors of bone, telomere length correlated with roentgenographic grade due to frequency of cell division, and high telomerase activity indicated the aggressiveness. In desmoids tumors, decreasing telomere length correlated with tumor size due to increased duration of proliferation in the tumor, and tumor aggressiveness. In sarcomas, ALT is a very significant prognostic risk factor for sarcoma patients. In ALT-negative sarcoma patients, telomerase expression is a significant prognostic risk factor. *Summary:* Although recent studies indicate a positive correlation between the telomere maintenance mechanism and tumor aggressiveness in mesenchymal tumors, there are different and controversial data among previous reports. Therefore, further study is necessary to clarify telomere maintenance mechanisms in bone and soft tissue mesenchymal tumors.

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PYRUVATE KINASE TYPE M2: A TARGET OF DIFFERENT ONCOGENES WITH HIGH THERAPEUTIC POTENTIAL

S. Mazurek[1,2], W. Zwerschke[3], U. Rapp[4], J.L. Rosa[5] and E. Eigenbrodt[1]

[1]Institute of Biochemistry and Endocrinology, Veterinary Medicine, University of Giessen;
[2]ScheBo Biotech AG, Giessen, Germany;
[3]Institute for Biomedical Aging Research of the Austrian Academy of Sciences, Innsbruck, Austria;
[4]Institute for Medical Radiation and Cell Research, University of Würzburg, Germany;
[5]Department of Physiological Sciences, University of Barcelona, Hospitalet del Llobregat, Spain

Pyruvate kinase catalyzes the last step within glycolysis, the dephosphorylation of phosphoenolpyruvate (PEP) to pyruvate, and is responsible for net ATP production within the glycolytic pathway. Tissues with different metabolic functions express different isoenzymes of pyruvate kinase (type M1, M2, R or L, respectively). All proliferating cells, *i.e.* normal proliferating cells, embryonic cells, adult stem cells and tumor cells in particular, express pyruvate kinase isoenzyme type M2 (M2-PK, PKM2), which plays a key role in the regulation of the Warburg effect. M2-PK may occur in a highly active

tetrameric form, with a high affinity to its substrate PEP, which favors the breakdown of glucose to pyruvate and lactate with production of energy. It may also exist in a nearly inactive dimeric form with a low PEP affinity, which channels glucose carbons into synthetic processes, which debranch from glycolytic intermediates, *i.e.* nucleic-acid, amino acid and phospholipid synthesis. In tumor cells, the dimeric form of M2-PK, termed Tumor M2-PK, is always predominant and is favored by direct interaction with various oncogenes. The E7 oncoprotein of the human papilloma virus type 16 binds to M2-PK. pp60v-src kinase, the transforming principle of the Rous sarcoma virus, phosphorylates M2-PK in tyrosine. A-Raf kinase induces an increase in serine phosphorylation of M2-PK. The effect of A-Raf on the quaternary structure of M2-PK is secondarily influenced by the amino acid metabolism (glutamine, serine and alanine) of the individual cell line. The physiological function of the interaction of M2-PK with HERC 1 is still unknown.

However, the tetramer: dimer ratio of M2-PK may fluctuate in tumor cells depending upon the concentrations of signal metabolites, *e.g.* fructose 1,6-P2 which induces a re-association of the dimeric form of M2-PK to the tetrameric form. The M2-PK isoenzyme, with its ability to switch between a highly active tetrameric and inactive dimeric form, is an important metabolic sensor which adapts tumor metabolism to varying nutrient supply. Peptide aptamers which specifically bind to M2-PK and not the M1-PK isoenzyme (96% homology) were found to obstruct re-association of M2-PK to the tetrameric form thereby reducing ATP levels and tumor cell proliferation. (www.metabolic-database.com).

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PYRUVATE KINASE TYPE M2: AN IMPORTANT REGULATOR OF THE WARBURG EFFECT WITH HIGH DIAGNOSTIC POTENTIAL

S. Mazurek[1,2], P.D. Hardt[3], H.W. Wechsel[4], G. Fischer[5], D. Lüftner[6], G. Schulze[7] and J. Schneider[8]

[1]ScheBo Biotech AG, Giessen;
[2]Institute of Biochemistry and Endocrinology, Veterinary Medicine, University of Giessen,
[3]Third Medical Department, University Hospital Giessen;
[4]Department of Urology, Reinard-Nieter-Hospital, Wilhelmshaven;
[5]Institute for Pathology, Reinhard-Nieter-Hospital, Wilhelmshaven;
[6]Medical Clinic II, University Hospital Charité, Berlin,
[7]Institute for Laboratory Diagnosis, Helios Hospital, Berlin;
[8]Institute for Occupational and Social Medicine, University of Giessen, Germany

Proliferating cells - particularly tumor cells - express a special isoenzyme of pyruvate kinase termed type M2 (M2-PK, PKM2) which plays a key role in the regulation of the glycolytic flux rate and the Warburg effect. Pyruvate kinase catalyzes the dephosphory-lation of phosphoenolpyruvate (PEP) to pyruvate and is responsible for net ATP production within the glycolytic pathway. M2-PK may occur in a highly active tetrameric form and a nearly inactive dimeric form. The tetramer:dimer ratio of M2-PK determines whether glucose carbons are converted to pyruvate and lactate with the production of energy (tetrameric form), or are channeled into synthetic processes which debranch from glycolytic intermediates, such as nucleic acid, amino acid and phospholipid synthesis. (http://www.metabolic-database.com).

In tumor cells, M2-PK was found to be mainly in the dimeric form which has therefore been termed Tumor M2-PK. Immunohisto-logical studies of various tumors with monoclonal antibodies (Clone DF4, ScheBo Biotech, Giessen) which specifically recognize the dimeric form of M2-PK revealed a heterogeneous distribution of Tumor M2-PK in primary tumors, whereas their metastases are always characterized by homogeneously large amounts of Tumor M2-PK. The dimeric form of M2-PK is released from tumors into the patients' blood, most likely by tumor cell necrosis.

To quantify Tumor M2-PK in EDTA-plasma samples, a sandwich ELISA (ScheBo Biotech, Giessen) based on two monoclonal antibodies which specifically recognize the dimeric form of M2-PK has been developed. In accordance with the immunohistological results an increase in Tumor M2-PK in EDTA plasma samples, and a correlation with tumor stage, was found for renal cell carcinoma, thyroid, lung, breast, cervical, ovarian, pancreatic, gastric and colorectal cancer, as well as melanoma. When taken together these results show that Tumor M2-PK is an organ-unspecific marker, which reflects the metabolic activity of the tumors. A major application for Tumor M2-PK in EDTA plasma is patient follow-up to monitor success, failure and relapses during therapy.

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IMMUNOLOGICAL SIMILARITIES BETWEEN CANCER PATIENTS AND CHRONIC FATIGUE SYNDROME PATIENTS

Mira Meeus[1,2] and Jo Nijs[1,2]

[1]Department of Human Physiology, Faculty of Physical Education and Physiotherapy; Vrije Universiteit Brussel (VUB);
[2]Division of Musculoskeletal Physiotherapy, Department of Health Sciences, University College Antwerp (HA), Belgium

Background: Cancer and chronic fatigue syndrome (CFS) are both characterised by fatigue and severe disability. Fatigue is the hallmark of CFS, and fatigue is a common symptom experienced by people undergoing treatment for cancer. Although cancer is a well-known and recognized disease and

CFS is rather unknown and underestimated, the fatigue is not the only link between the two diseases. By comparing the two diseases we might gain more insight into the physiopathology and the cause of some common complaints.
Methods: Based on our own findings and on literature results the intracellular immune dysfunctions in CFS will be explained, the hypothesised relation with fatigue will be elucidated and finally the link with cancer patients will be made. *Results:* Both in cancer and in CFS, the immune system has shown important and partly overlapping changes. One of the major intracellular immune dysfunctions in CFS is the deregulation of the RNase L antiviral pathway. This deregulation may lead to channelopathies that in turn might be responsible for some of the CFS symptoms. Also in cancer patients abnormalities in the RNase L pathway are already documented. The R462Q variant of RNase L, having about 3-fold reduced catalytic activity *in vitro*, is the most prevalent genetic marker for prostate cancer. Malfunctioning of natural killer (NK) cells (*i.e.* decreased natural killer cell cytotoxicity) has long been recognised as an important factor in the development and reoccurrence of cancer, and has been documented repeatedly in people with CFS. Besides these major interfaces disturbed apoptotic mechanisms and oxidative stress or excessive nitric oxide may play a role in the two diseases and in the common symptom fatigue. With regard to these immunological abnormalities prudence is called for during the rehabilitation of these patients. Exercise is currently recommended as a conservative intervention for both fatigued cancer patients and CFS patients, but based on literature findings and our own results we know that too vigorous exercise may worsen the immune system and the complaints in CFS patients. The exercise response to intensive activity in cancer patients is less understood. On the other hand, it is well known that moderate exercise has beneficial effects on the immune system and may even have preventive effects on cancer in all disease stages. Therefore physical rehabilitation should be carefully balanced. *Conclusion:* Despite the high mortality rates in cancer, cancer and CFS present several similarities. The immunological abnormalities, such as a deregulated RNase L pathway, reduced NK cytotoxicity, increased oxidative stress etc are present in both diseases. These anomalies may be responsible for some of the common complaints, like fatigue, and should be considered in the physical rehabilitation of these patients.

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CURRENT STANDARDS IN PSYCHOSOCIAL CANCER CARE

Anja Mehnert

Department of Medical Psychology, University Medical Center Hamburg-Eppendorf, Martinistr. 52 – S35, 20246 Hamburg, Germany

Over the past thirty years, psychosocial cancer care has become an integral part of cancer treatment in many countries around the world, a development that has been accompanied by research findings and meta-analyses showing that psychosocial care enhances the well-being and quality of life of cancer patients. This increasing integration is reflected in the rising number of new institutions for psychosocial care, the increase in research programs, the large number of scientific publications and conferences, as well as in the increased acceptance of psychosocial oncology among medical professions, cancer patients and the general public.

First approaches to offer cancer patients psychological help in various European countries started in the middle of the 1970s. Experience in these approaches demonstrate that in most cases major problems in understanding and acceptance arose. Medical doctors and nurses expressed their critical attitudes towards psycho-oncology. Only very few clinical institutions reported a successful implementation of psychosocial interventions. There were various reasons for the problems of implementing psychosocial services into clinical practice. Knowledge about the relevance of psychological and social factors for the development and the course of diseases such as cancer were not as widespread as today among health care professionals. Thus, the understanding of disease was strongly determined by biological models. Furthermore, medical health care professionals often showed a lack of willingness to let new professions such as psychologists or psychotherapists participate within the care of patients.

Since then, the situation has substantially changed. Comprehensive models of diseases and treatment are prevalent among the general population, as well as among health care professionals. Psycho-oncological interventions and programs have gained increasing interest, addressing various aspects of cancer survivorship such as psychological comorbidity, psychosocial care needs and return to work in cancer survivors. One major factor that contributes to this development is the well-established psycho-oncological research. Nevertheless, psycho-oncological services are not well implemented in many health care institutions and facilities for cancer patients. Today, as important as the above mentioned historical barriers is the lack of personal and financial resources to meet the psycho-oncological needs of cancer patients.

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PATIENT-TO-PATIENT AND INTRA-PATIENT SUB-SAMPLING VARIATION IN GENE EXPRESSION PROFILES IN NSCLC

M. Meister[1], A. Belousov[5], E.C. Xu[1], P. Schnabel[4], A. Warth[4], H. Hoffmann[2], H. Dienemann[2], H. Bodenmueller[5], W. Zolg[5], F.J.F. Herth[3] and T. Muley[1]

[1]Translational Research Unit;
[2]Department of Surgery;

[3]Department of Pulmonary Medicine, Thoraxklinik, University of Heidelberg;
[4]Institute of Pathology, University of Heidelberg;
[5]Roche Diagnostics, Penzberg, Germany

We assessed the influence of tumor cell composition on gene expression in non-small cell lung cancer (NSCLC) analyzing patient-to-patient and intra-patient variations. Four different sites of individual tumors from 20 patients undergoing resection for primary lung cancer were used to establish the gene expression pattern captured by Affymetrix HG-U133 Plus 2.0 arrays. Inter-patient (patient-to-patient) and intra-patient (tumor site to tumor site) variability of gene expression were determined in an uni-variant and multi-variate setting using linear mixed effect model approach and partial least squares regression, respectively. The majority of probe sets revealed consistently larger differences in expression among the patients than seen within the different tumor sub-samples of the same patient. Considering the 0.6% of the probes sets which showed sensible tumor site variability, gene ontology analysis revealed that these genes were 'at random'. No association to TNM status, tumor stage, patient gender/age, or other study co-variates was detected. A small number of genes were identified to be associated with tumor cell or stroma tissue content. In conclusion, expression profiles based on single tumor tissue pieces from NSCLC patients are representative of the entire tumor, if viable tumor cell content is equal to or greater fifty percent.

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ATHEROSCLEROSIS AND CANCER: A COINCIDENCE OR A CAUSAL LINK?

Bohuslav Melichar, Hana Kalábová and Hana Študentová

Department of Oncology, Palacký University Medical School and Teaching Hospital, Olomouc, and Department of Oncology and Radiotherapy, Charles University Medical School and Teaching Hospital, Hradec Králové, Czech Republic

Cancer and the complications of atherosclerosis represent two leading causes of death in the industrialized nations. Cancer and atherosclerosis share risk factors and commonly affect the same individuals. A causal link in the pathogenesis of these two disorders that would exist in addition to the shared risk factors (*e.g.* age or smoking) is a matter of dispute. Inflammatory response, both local and systemic, plays an important role in the progression of both atherosclerosis and cancer. For example, systemic inflammatory response resulting from chronic infections that is reflected in increased serum concentrations of C-reactive protein has been postulated to play an important role in the progression of atherosclerosis. Advanced/metastatic cancer is also commonly associated with systemic inflammatory response and elevation of serum C-reactive protein, but poor prognosis of patients with advanced/metastatic cancer might have prevented the clinical manifestation of accelerated atherosclerosis in this group of patients. The progress in the therapy of some tumors in the past decades may lead to unmasking of the effect of systemic inflammatory response associated with advanced/metastatic cancer on the progression of atherosclerosis. Moreover, the toxicity of anticancer therapy may result in the progression of atherosclerosis. The clinical data so far indicate increased incidence of cardiovascular disorders in survivors of childhood cancer or germ-cell tumors that is associated with a history of chemotherapy. The data on more common adult tumors are more scarce. Hormonal therapy may have an adverse effect on the risk factors of atherosclerosis. However, the adverse effect of aromatase inhibitors, now commonly used in the adjuvant therapy of breast carcinoma, on serum lipid concentrations may reflect the effect of tamoxifen withdrawal rather than the action of aromatase inhibitors themselves. More pronounced adverse effects on the progression of atherosclerosis could be expected from the use of drugs targeting the vascular endothelial growth factor or its receptors, but these drugs were introduced only recently and are now used almost exclusively in patients with incurable metastatic tumors. In conclusion, we are just beginning to appreciate the extent to which advanced/metastatic cancer and/or anticancer therapy causes progression of atherosclerosis. With improved therapy of more common tumors, a causal link between cancer and atherosclerosis mediated by inflammatory response may become obvious. The impact of new biologic agents on the progression of atherosclerosis has yet to be determined.

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CHEMOTHERAPY RESISTANCE IN GLIOMAS

M. Mellai, L. Annovazzi, V. Caldera, E. Andreoli and D. Schiffer

Neuro-bio-oncology Center of Policlinico di Monza/University of Turin, Vercelli, Italy

Tumor cell resistance to radio – and chemotherapy is the major bias in tumor treatment. The mechamisms are today topics of intense study. In its clinical applications, MGMT (O^6 - methylguanine DNA methylatransferase) is very important. Promoter hypermethylation of the MGMT gene blocks the removal of methyl groups from the O^6 position of guanine produced by alkylating agents. Methylated tumors show better outcome in comparison with unmethylated ones. Methylation can be assessed by Methylation Specific PCR (MSP) or by immunohistochemistry and it is used today in association with Temozolomide.

In the absence of functional MGMT, an important additional mechanism of resistance to alkylating agents is DNA Mismatch Repair (MMR) deficiency. This pathway functions as a mismatch excision repair leading to additional DNA double strand breaks and apoptotic cell death in cells lacking MGMT activity.

Alternative mechanisms involve Nucleotide and Base Excision Repair pathways (NER and BER, respectively). The latter refers to the Poly(ADP-ribose) polymerase-1 (PARP-1) enzymes which are activated by DNA damage.

The ATP-Binding Cassette (ABC) genes are responsible for the multidrug resistance (MDR) to the topoisomerase inhibitors VP-16 as well as to vincristine in a number of cancers. Our experience in gliomas is discussed.

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RNA AND DNA QUADRUPLEXES

Jean-Louis Mergny

INSERM U565, CNRS UMR 5153, Muséum National d'Histoire Naturelle USM 503, 43 rue Cuvier, 75005 Paris, France

Evidence is growing that unusual nucleic acid structures called G-quadruplexes (G4) play important biological roles, either at the DNA or RNA level. G-quadruplexes result from the hydrophobic stacking of guanine quartets (Figure); each quartet being a planar association of 4 guanines (or guanine analogs (1)) held together by 8 hydrogen bonds. A cation (typically K^+) is located between two quartets and forms cation-dipole interactions with 8 guanines.

I will present some of the properties and applications of G4 structures in areas ranging from nanotechnology (2), biotechnology to medicinal chemistry. Two quadruplex-related compounds (a G-rich oligonucleotide and a G4 ligand) are currently in phase II clinical trials. G4 structures are very stable under physiological conditions and it is likely that nature designed proteins such as helicases or chaperones to control their formation.

Quadruplexes may be involved in telomere regulation. The 3' G-rich telomeric overhang from a variety of species may adopt a G-quadruplex structure. We analyzed several series of G4 ligands that recognize the human telomeric quadruplex and were initially considered as telomerase inhibitors. However, we demonstrated that inhibitory effect of such molecules has been overestimated and that G4 ligands rather constitute a different class of biologically active compounds (3, 4). Finally, RNA sequences may also form quadruplexes. The transcribed telomeric repeats (TERRA) and the RNA component of telomerase (called hTERC or hTR) may form very stable intramolecular guanine quadruplexes (5).

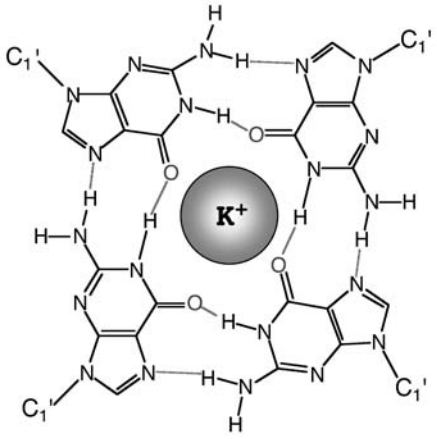

Figure. *Presentation of a G-quartet, with four coplanar guanines.*

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RELATIONSHIP BETWEEN PSYCHONCOLOGY AND PSYCHONEUROENDOCRINOIMMUNOLOGY (PNEI): EVALUATION OF ANTICANCER IMMUNITY IN RELATION TO THE PSYCHOSPIRITUAL STATE OF CANCER PATIENTS

Giusy Messina

Psychological Service, Policlinico Maggiore Hospital, University of Milan, Italy

It is long known that the psychospiritual status may influence tumour growth and the prognosis of the neoplastic disease. However, only with the development of psychoneuro-endocrinoimmunology (PNEI) has it been possible to characterize the neuroimmunochemical mechanism responsible for the influence of the psychospiritual condition on tumour growth, namely through the modulation of the immune system, which appears to be able to control cancer

cell proliferation. Anticancer immunity is stimulated by IL-2 and IL-12, whereas it is inhibited by IL-10 and IL-6. According to recent advances in tumour immunobiology, at present the most important cell suppressing the anticancer immunity is the T regulatory lymphocyte (T reg), which may identify CD4+CD25+ cells. At present it is possible to monitor the clinical history of a neoplastic disease by evaluating not only tumor marker concentration but also the immunobiological status of cancer patients by measuring immune parameters related to anticancer immunity, such as IL-2, IL-12, lymphocyte count and the different lymphocyte subpopulations. In particular, it has been observed that low IL-6 and IL-10, a low number of lymphocytes and T-helper lymphocytes (CD4) and an increased number of T reg is associated with poor prognosis. Stress, chronic pain, depression and anxiety, which have a negative influence on the prognosis of cancer, appear to act through the opioid system, whose activation may suppress anticancer immunity. On the contrary, pleasure and spirituality may stimulate the anticancer immunity through the activation of the pineal gland and the brain endocannabinoid system. Thus PNEI may allow us to explain the overall clinical evidence shown by psycho-oncological studies. However within the great number of psychological variables, spirituality has to be considered as being different from the psychological profile. The spirituality would act through the pineal gland and the brain endocannabinoid system, whereas the psychological status would be mediated by the opioid system. Several tests have been used to investigate the psychological profile of cancer patients but according to our experience the most appropriate psychological examination could be represented by the Rorschach's test, because of rational objectivity. In contrast, at present the investigation of spirituality remains difficult, since most questionnaires proposed up to now are generally limited to the investigation of religious practice. We have performed several studies to investigate the different psychological profile of cancer patients in relation to their psychoneuroendocrinoimmune status. The psychological profile was investigate by the Rorschach's test and the spiritual status was analyzed by a special spiritual test. We performed five main studies, obtaining the following relationships: i) low numbers of lymphocytes and T-helper lymphocytes (CD4) in patients was associated with solid tumour and suppression of spiritual and sexual sensitivity; the study was carried out in 60 patients, 30 of them showed a metastatic disease. Colon, lung and breast cancer were the most frequent tumours in our patients. ii) Low efficacy of IL-2 immunotherapy and low lymphocyte response to IL-2, occurring in cancer patients, were associated with anxiety and/or loss of sexual identity. The study was performed in 30 patients with renal cancer. iii) A low number of lymphocytes was associated with alteration in the circadian rhythm of cortisol and hypercortisolemia in cancer patients with a low spiritual profile. The study was carried out in 30 patients with metastatic non-small cell lung cancer. iv) An abnormally high percent of T-regulatory lymphocytes (CD4CD25) in patients with solid tumour was associated with a self-punishment' status. The study was carried out in 40 patients with locally limited or metastatic neoplasm. v) Lack of surgery induced-hyperprolactinemia in operable breast cancer patients was associated with suppression of the maternal behaviour. The study was performed in 30 women.

These results would suggest the possibility to investigate the psychoneuroimmune basis of the overall psychological profile, characterizing cancer patients during the clinical history of their disease. Further studies by investigating the brain endocannabinoid system through the detection of the blood concentration of the main endocannabinoid agent, anandamide, will clarify the neurochemical alteration responsible for the progressive loss of pleasure occurring during neoplastic disease.

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TREATMENT AND COFACTOR STUDIES FOR HUMAN PAPILLOMAVIRUS-ASSOCIATED CERVICAL CANCER

Samina Alam, Brian Bowser and Craig Meyers

Department of Microbiology and Immunology, H107, Pennsylvania State University College of Medicine, Hershey, PA. 17033 USA

One of the major goals of our laboratory is to identify factors and their inherent targeting mechanisms which affect progression of human papillomavirus (HPV)-associated cervical cancers. Two types of cofactor studies are currently underway in our laboratory which have addressed these issues. The first set of studies deals with the development of therapeutics for cervical as well as other types of cancer. Epidemiological studies indicate a negative correlation between adeno-associated virus type 2 (AAV2) infection and the incidence of HPV associated cervical cancer. The oncosuppressive properties of AAV2 is linked to the ability of this virus to perturb cell-cycle progression by decreasing cellular proliferation rates and mediating growth arrest. One of the hallmarks of HPV infection is the abrogation of key cell cycle regulatory proteins. We utilized HPV infected human keratinocytes as well as normal human foreskin keratinocytes, which are natural hosts for both AAV2 and HPV. Cultures co-infected with HPV31b and AAV2 displayed reduced $p16^{INK4}$, $p21^{WAF1}$ and $p27^{KIP1}$ CDK inhibitor protein levels culminating in apoptotic cell death, as determined by DNA laddering. Cell death could be correlated with an increased percentage of cells with S-phase DNA content, concurrent with AAV2 Rep protein expression. Simultaneously, pRb protein levels were stabilized in its hypophosphorylated form and HPV E7 oncoprotein

expression was severely diminished. In addition, other cell lines infected with different HPV types from both low- and high-grade lesions were also sensitive to AAV2-induced cell death. Interestingly, HPV-negative carcinoma lines, including a squamous cell carcinoma line, the MCF-7 breast cancer line, and the PC3 prostate cancer line, also underwent apoptotic cell death following infection with AAV2. In sharp contrast, primary foreskin keratinocytes infected with AAV2 were resistant to apoptosis. The ability of AAV2 to exclusively target deregulated cells indicates its potential for being developed as an anticancer agent in gene therapy protocols.

The second set of studies is also based on epidemiological studies which suggest that HPV-infected women who smoke face an increased risk for developing cervical cancer. We are the first laboratory to demonstrate a molecular link between exposure of HPV-infected tissue to cigarette smoke carcinogens and its effect on the viral life cycle. Benzo[*a*]pyrene (B*a*P) is a well-characterized cigarette smoke carcinogen and has been found to be concentrated in the cervical mucus. HPV genome amplification was enhanced at low concentrations of B*a*P, whereas higher concentrations induced significant increases in the synthesis of infectious virus. We have also studied host cell cycle and differentiation gene expression, and have correlated changes to the increase in viral genomes and production of infectious viral particles. Since most HPV infections are spontaneously cleared, our data suggests that B*a*P induction of increased virion synthesis and genome amplification give rise to an increased viral load, affecting the persistence of the viral infection and level of oncogene expression.

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METHYLATION PROFILE OF hMLH1, MGMT, APC AND CDH1 GENES IN GREEK PATIENTS WITH COLON ADENOCARCINOMA

Christina I. Michailidi[1], Stamatios E. Theocharis[2], Gregorios P. Kouraklis[3], Paraskevas Katsaronis[3] and Constantinos Troungos[1]

[1]Department of Biological Chemistry, Medical School, University of Athens; Athens; [2]Department of Forensic Medicine and Toxicology, Medical School, University of Athens; Athens; [3]Second Department of Propedeutic Surgery, Medical School, University of Athens; Athens, Greece

Introduction: Colon cancer is a common malignancy usually arising from a benign neoplasm which is developed into adenocarcinoma through a certain histological progression sequence. Genomic instability and epigenetic alterations - with hypermethylation of cytosine in CpG islands in the promoter of certain genes- play an important role in carcinogenesis and tumour progression of this type of cancer. Epigenetic changes conduce to cancer formation through the transcriptional silencing of certain genes. The aim of this study was to analyze the promoter methylation of genes related with mismatch repair as hMLH1; repair genes like MGMT which interferes in the repair of damages in O^6 position of guanine by removing alkyl groups; tumour suppressor genes as APC and possible tumour suppressor genes like CDH1 which encodes epithelial cadherin preventing cells from growing and dividing in an uncontrolled way to form a malignant tumour. *Materials and Methods:* Genomic DNA was isolated from 28 colon cancer patients (14Male/14Female), with mean age 64 years. DNA was then subjected to chemical modification with sodium bisulfate and followed by Methylation Specific PCR (MSP) amplified by primer pairs specific for the methylated and unmethylated sequences. PCR products were analyzed on 2% agarose gel, stained with ethidium bromide and visualized in UV. The methylation results were associated with patients' gender and age, tumours' histological grade and stage. *Results:* Promoter methylation for hMLH1 gene was observed in 1 out of 28 (3%), for MGMT in 15 out of 28 (53%), for APC in 5 out of 28 (18%) and for CDH1 in 24 out of 28 (86%). In 15 out of 28 (61%) cases methylation in 2 or more of the examined genes was noted. Methylation profiles were also associated with clinicopathological characteristics of the colon cancer patients. A statistically significant association between methylation of CDH1 gene and Dukes' stage (stage A, B, C *vs*. D; p=0.002) was found. Moreover, an association (although not statistically significant) between methylation status in two or more genes with either tumour histological grade (well and moderately *vs*. poorly differentiated) or Dukes' stage (stage A, B, C *vs*. D) was also noted (p=0.055 and p=0.063, respectively). *Conclusion:* Our data confirmed alterations in the methylation status of hMLH1, APC and MGMT genes in colon cancer, although no significant association was noticed with any of the examined clinicopathological parameters. Additionally, the promoter of CDH1 gene was found to be very frequently methylated, being related with Dukes' stage. Furthermore hypermethylation in two or more genes seemed to be related with tumour characteristics. By expanding our research effort in the examination of methylation status during the whole procedure from normal tissue to early adenoma and carcinoma formation, we may further delineate whether methylation can be used as a possible target therapy in colon cancer, since demethylation can restore gene expression.

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FOCAL ADHESION KINASE (FAK) EXPRESSION IN HUMAN BENIGN AND MALIGNANT THYROID LESIONS

Christina Michailidi[1], Nikolaos Chatzizacharias[1], Vassilios Stolakis[1], Paraskevi Alexandrou[2], Jerzy Klijanienko[3], Ioanna Delladetsima[2] and Stamatios Theocharis[1,3]

[1]Department of Forensic Medicine and Toxicology, University of Athens, Athens;
[2]First Department of Pathology, Medical School, University of Athens, Athens, Greece;
[3]Department of Pathology, Institut Curie, Paris, France

Background: Focal adhesion kinase (FAK), a non-receptor tyrosine kinase protein, acts as an early modulator of integrin signaling cascade, regulating basic cellular functions. In transformed cells unopposed FAK signaling has been considered to promote tumor growth, progression and metastasis. The aim of the present study was to evaluate the clinical significance of FAK expression in human benign and malignant thyroid lesions. *Design:* FAK protein expression was assessed immunohistochemically in formalin-fixed, paraffin-embedded thyroid tissues from 116 patients with benign (46 hyperplastic nodules and 9 Hashimoto thyroiditis) and malignant (49 papillary, 6 medullary, 5 follicular and 1 anaplastic thyroid carcinomas) lesions. Immunohistochemical evaluation was based on both staining intensity and the amount of positive stained cells within thyroid lesion, graded in a two step scale as weak or no staining and moderate or strong staining. Statistical analysis was performed to compare the expression of FAK protein between benign and malignant thyroid lesions, as well as between Hashimoto thyroiditis and hyperplastic thyroid lesions within benign subgroup. In the subgroup of malignant thyroid tumors, FAK protein expression was statistically analyzed in relation to TNM stage and tumor proliferative capacity, assessed by Ki-67 labeling index. *Results:* FAK protein expression was characterized as weak or no staining in 83 (72%) and moderate or strong staining in 33 (28%) out of 116 cases. In malignant thyroid lesions, FAK protein expression was characterized as weak or no staining in 35 (57%) and moderate or strong staining in 26 (43%) out of 61 cases. In benign thyroid lesions, FAK protein expression was characterized as weak or no staining in 48 (87%), moderate or strong staining in 7 (13%) out of 55 cases. The level of FAK protein expression was statistically significantly higher in malignant thyroid lesions compared with benign ones (p=0.001). In malignant thyroid lesions, FAK protein expression was statistically significantly associated with the presence of nodal metastasis (p=0.047). Additionally, a borderline association between FAK protein expression and tumor cells' proliferating capacity was noted (p=0.065). Regarding the benign thyroid lesions, FAK protein expression was not statistically significantly associated with histological type, in order to provide a distinct discrimination between Hashimoto thyroiditis and hyperplastic nodules. *Conclusion:* The current data confirm the expression of FAK protein in thyroid lesions that can significantly distinguish benign from malignant cases, being also associated with disease stage (N) and proliferative capacity in thyroid malignant cells. Further studies are needed in order to delineate the clinical importance of FAK expression in thyroid neoplasia and its possible use as a target for future therapy applications.

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ANTICANCER PROPERTIES AND MDR MODULATORY EFFECT OF PLANT POLYPHENOLS AND THEIR INTERACTION WITH MEMBRANE COMPONENTS

Krystyna Michalak[1], Olga Wesołowska[1], Jerzy Wiśniewski[1], Kamila Środa[1], Andrzej Teisseyre[1], Michał Kuždžał[1], Janez Štrancar[2], Noélia Duarte[3] and Maria-José U. Ferreira[3]

[1]Department of Biophysics, Wrocław Medical University, ul. Chałubińskiego 10, 50368 Wrocław, Poland; [2]Laboratory of Biophysics, Jozef Stefan Institute, Jamova 39, Ljubljana, Slovenia;
[3]CECF/iMed. UL, Faculty of Pharmacy, University of Lisbon, Av. das Forças Armadas, 1600-083 Lisbon, Portugal

Many compounds of plant origin have been identified to be interesting for potential application in cancer prevention or chemotherapy. Such agents were found among flavonoids and stilbenes. In broad *in vitro* and *in vivo* studies, the ability of plant-derived polyphenols to interact with different cellular targets was revealed. Resveratrol (trans-3,4,5'-trihydroxystilbene) influences many cellular signaling pathways and affects all three stages of carcinogenesis. In this work the effect of several stilbenes (resveratrol, its analogue piceatannol, and piceatannol derivatives) and some flavonoids on activity of ABC multidrug transporters and potassium channels Kv1.3 was examined. Recently the role of potassium channel activity in cancer has emerged. Several of the compounds studied were found to be potent potassium channel inhibitors. Strong inhibition of membrane transporters and ion channels was often observed for hydroxy-, methoxy-, acetoxy- and prenyl- derivatives in relation to the parent compounds. Piceatannol, naringenin and some of their derivatives were the most active inhibitors of MRP1 multidrug transporter. The influence of plant polyphenols on integral membrane proteins such as MDR transporters and ion channels can be mediated at least in part by non-specific membrane effects. Flavonoids and stilbene interactions with lipid bilayers was determined by fluorescence and ESR spectroscopy. Simulation of the

experimental ESR spectra and application of GHOST condensation method were applied to study the effect of polyphenols on membrane domain structure. The significance of interaction of studied phytochemicals with membrane transporters, channels and lipid bilayer for their anticancer properties was discussed.

Antiproliferative properties of the compounds were tested in sensitive and doxorubicin-resistant, P-gp overexpressing colon cancer cell lines LoVo and LoVo/Dx, respectively. Tangeretin, natural polymethoxylated flavone and 8-prenylnaringenin most effectively inhibited cell growth both in sensitive and resistant cancer cell lines. Antiproliferative action was compared with pro-apoptic properties of the compounds.

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SEX STEROID-ACTIVATED NON GENOMIC SIGNALLING AS A TARGET FOR HORMONE DEPENDENT CANCER THERAPY

A. Migliaccio, G. Castoria, L. Varricchio, A. de Falco, M. Di Domenico and F. Auricchio

Department of General Pathology. II University of Naples. Via L. De Crecchio, 7 – 80138 – Naples, Italy

In human mammary and prostate cancer cells, steroid hormones and EGF trigger association of the androgen receptor (AR)-estradiol receptor (ER-α or -β) complex with Src. This interactions activates Src and drives the cell into the G1-S phase progression. This suggests that such an interaction can be targeted to control cancer cell growth.

In view of this, we identified the AR sequence responsible for the androgen receptor/Src interaction and synthesized a 10 amino acid peptide derived from this sequence, which inhibits this interaction. Treatment of human prostate or mammary cancer cells (LNCaP or MCF-7, respectively) with nanomolar concentrations of this peptide prevented the androgen- or estradiol-induced association between AR or ER and Src. The peptide also inhibited the Src/Erk pathway, cyclin D1 expression and DNA synthesis in steroid-stimulated LNCaP and MCF-7 cells, without interfering with receptor-dependent transcriptional activity. Similarly, the peptide prevented the S-phase entry of LNCaP and MCF-7 cells treated with EGF as well as mouse embryo fibroblasts stimulated with androgen or EGF. Interestingly, the peptide did not inhibit S-phase entry or cytoskeletal changes induced by EGF treatment of AR-negative prostate cancer cell lines. The interest of these findings is highlighted by the fact that the peptide strongly inhibited the growth of LNCaP xenografts established in nude mice.

The AR/ER/src association induced by steroid hormones and EGF was also abolished in whole cells and *in vitro* by a six amino acid peptide, which mimics the sequence around the phosphotyrosine residue in position 537 of ER-α. This sequence is homologous to that surrounding the phosphotyrosine 443 of ERβ. The phoshorylated peptide, at nanomolar concentrations, hindered ER/Src interaction and inhibited Src/Erk pathway, cyclin D1 expression and DNA synthesis induced by estradiol, or androgen, or triggered by EGF in MCF-7 and LNCaP cells. In contrast, no inhibition of the Src-mediated EGF action on DNA synthesis was detectable in human mammary cancer cells which did not express ER (MDA-MB231), indicating that this peptide specifically targets the ER-associated Src. Like the AR-derived peptide, the ER-derived peptide, in contrast to classical steroid antagonists, did not interfere in ER- or AR-dependent transcriptional activity. Nevertheless, it markedly inhibited the growth of MCF-7 cell xenografts induced in estradiol-treated nude mice. Both peptides represent the first example of specific inhibitors of steroid receptor-dependent signal transducing activity. More importantly, these findings suggest that inhibition of association of steroid receptors with Src or other signalling effectors may have therapeutic applications for patients with hormone-dependent tumors.

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THERAPEUTIC AND DIAGNOSTIC APPLICATIONS OF APTAMERS IN CANCER

S. Missailidis

Department of Chemistry and Analytical Sciences, The Open University, Walton Hall, Milton Keynes, MK5 7AS, UK

Aptamers were first described in 1990, and during the past 18 years they have found a number of applications, as research tools or as therapeutic and diagnostic entities in the fight against disease.

Aptamers are single stranded RNA or DNA molecules selected through evolutionary processes for high binding affinity and exceptional specificity against a variety of targets, including enzymes, cell surface receptor proteins, cytokines and chemokines, antibodies, as well as small organic compounds. Although the majority of work on aptamers has not focused on cancer, one of the early aptamers against the VEGF has recently received FDA approval against Macular Degeneration, as a PEGylated preparation, and has been in clinical trials against tumour angiogenesis. Aptamers against novel angiogenesis targets have also been prepared by our group and are currently in preclinical testing. Some aptamers have demonstrated direct therapeutic effect due to their apoptotic potential, resulting from blocking vital cellular pathways. The aptamer against nucleolin, a dimeric G-quadruplex oligo, has shown great therapeutic potential in phase I clinical trials and has now entered phase II trials for treatment for acute myeloid leukemia and renal cancer. An aptamer against an epithelial

tumour cell marker developed by our group, has also shown great therapeutic potential on a 6 day treatment regime (results presented), as has an anti-HER3 aptamer. With the exception of the nucleolin aptamer, which is dimeric in nature, most therapeutic aptamers have been PEGylated, to increase their circulation time. These include the PDGF, the tenascin-C and our MUC1 aptamers, currently in various stages of clinical development. Furthermore, aptamers have unique properties as delivery vehicles and have been successfully coupled to small chemotherapy agents, such as the aptamers against neutrophil elastase, or to appropriate chelators loaded with radionuclides, exemplified by own MUC1 aptamer work. The later can function both as imaging agents in medical imaging using gamma camera, allowing fast clearance from the system and good tumour localisation and penetration or PEGylated, as therapeutic radiopharmaceuticals when loaded with a beta emitter, such as Re188 in the treatment of breast and bladder cancer. Finally, the unique binding properties of aptamers are being utilised in the development of medical sensors, for targets such as PSA, MUC1 and others, where current detection limitations have restricted application.

Aptamers remain a complicated field in terms of intellectual property, with strong control of intellectual property by two US companies, which has deterred the pharmaceutical industry from actively developing in this field. However, as the field is about to open in the next few year, more and more aptamers are being developed therapeutically or diagnostically.

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DYSTROGLYCAN FUNCTION IN PROSTATE CANCER

Andrew Mitchell[1], Simon Cross[2], Freddie Hamdy[2] and Steve Winder[1]

Departments of [1]Biomedical Sciences and [2]Academic Urology, Section of Oncology, University of Sheffield, UK

Prostate cancer (PCa) is a significant cause of morbidity and mortality in the Western world. In its most aggressive forms, there is rapid growth and metastasis during which cells detach from the primary tumour and migrate within the blood and lymphatic system to form secondary tumours.

Dystroglycan (DG) is a ubiquitously expressed cell adhesion molecule that provides a link between the extracellular matrix and the actin cytoskeleton. It is important for cell motility, polarity and possibly signal transduction. We have examined the function, distribution and localisation of DG in several PCa cell lines including LNCaP, PC3 and DU145. We found that DG is extensively modified during increases in cell confluency *in vitro*, through both MMP-mediated proteolysis and changes in glycosylation pattern. Furthermore, anchorage-independent growth assays and Transwell invasion studies revealed that colony-forming and invasive capabilities of PCa cells are significantly affected by changes in DG levels. Reduced DG levels promoted growth in soft agar, whereas increased DG promoted Transwell invasion. Immunohistochemically, there was also a significant reduction in DG immunoreactivity within PCa primary tumour samples. However, in metastatic PCa samples from bone, in 5 samples with reduced DG levels in the primary tumour, DG levels were increased at the secondary site. DG would therefore appear to be important for maintaining normal epithelial structure. Reduction in DG levels possibly contribute to the early stages of cancer progression, however DG might need to be re-expressed in order for tumours to metastasise and establish at secondary sites. Further investigations will help better understand the complexities and role(s) of DG in PCa and also aid in elucidating the mechanism(s) and consequences of DG alteration in the disease.

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REGULATORY ROLE OF HUMAN AP-ENDONUCLEASE (APE1/REF-1) IN YB-1-MEDIATED ACTIVATION OF MULTIDRUG RESISTANCE (*MDR1*) GENE

Kishor K. Bhakat[1], Ranajoy Chattopadhyay[1], Soumita Das[1], Amit K. Maiti[1], Istvan Boldogh[1], Jingwu Xie[1], Tapas K. Hazra[1], Kimitoshi Kohno[2] and Sankar Mitra[1]

[1]Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX 77555, USA; [2]University of Occupational and Environmental Health School of Medicine, Iseigaoka, Kitakyushu, Japan

Resistance to multiple chemotherapeutic agents is the major failure for chemoprevention of cancer. Multidrug resistance often occurs due to enhanced expression of p-glycoprotein (p-gp)/MDR1, the product of multidrug resistance gene, *MDR1*. MDR1 belongs to a family of exporters which mediate efflux of a variety of xenobiotics including cancer chemotherapeutic drugs. MDR1 overexpression often observed in tumor cells contributes to failure of cancer chemotherapy. The mammalian AP-endonuclease (APE1/Ref-1), a central enzyme involved in repair of oxidative base damage and DNA strand breaks, has a second activity as a transcriptional co-regulator when it complexes with a number of transcription factors, including AP-1, p53 and NF-κB and regulates their function *via* redox-dependent and -independent mechanisms. APE1 overexpression, often observed in tumor cells, is associated with resistance to various anticancer drugs; its down-regulation sensitizes tumor cells to such agents. Because the drug resistance of many tumor cells could not be explained by APE1's DNA repair activity which can not repair the DNA lesions

induced by such drugs, APE1's transcriptional regulatory function must be involved in drug resistance. We have shown that APE1, preferably in acetylated form, stably interacts with the Y-box-binding protein-1 (YB-1), and enhances its binding to the Y-box element, leading to MDR1 activation. Enhanced MDR1 level due to ectopic expression of WT APE1, but not of its nonacetylable mutant, underscores the importance of APE1's acetylation in its co-activator function. APE1 down-regulation sensitizes MDR1-overexpressing tumor cells to cisplatin or doxorubicin, showing APE1's critical role in YB-1-mediated gene expression and thus drug resistance in tumor cells. A systematic increase in both APE1 and MDR1 expression was observed in non-small cell lung cancer tissue samples. Thus our study has established the novel role of acetylation-mediated transcriptional regulatory function of APE1, making it a potential target for drug sensitization of tumor cells.

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HOMOLOGOUS RECOMBINATION AND CHROMOSOME INSTABILITY IN CANCER

Kiyoshi Miyagawa

Laboratory of Molecular Radiology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

A double-strand break is a critical lesion that can promote genome instability. It is well established that double-strand breaks induced by ionizing radiation are mainly repaired by nonhomologous end-joining, whereas homologous recombination plays a critical role in the repair of replication-associated DNA breaks. Because defects in these pathways lead to chromosome aberrations ranging from translocations to aneuploidy, altered repair pathways are assumed to contribute to tumor initiation and progression. Rad51 and its associated proteins are major players at early stages of homologous recombination. Because the breast cancer susceptibility protein BRCA2 directly associates with Rad51, impaired homologous recombination is assumed to contribute to tumor development in BRCA mutation-associated cancer. In addition, Rad51 and BRCA2 are functionally associated with other protein complexes. Among the complexes, Rad51 paralogs are involved in homologous recombination by assisting Rad51 functions. A link of Rad51 paralog with chromosome instability has been proposed from the observations that the frequency of aneuploidy is increased in their mutant cells. To understand the mechanism underlying chromosome instability induced by defective homologous recombination, we have generated colon cancer cell lines in which each Rad51 paralog is deleted. This study revealed that impaired centrosome integrity plays a role in chromosome instability induced by Rad51 paralog mutations. Moreover, we show that the p53-dependent checkpoint plays a role in the maintenance of genome integrity in cells deficient in Rad51B or Rad51C but not in XRCC3, indicating functional differences in Rad51 paralogs.

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OVEREXPRESSION OF *HER-2/NEU* PROTO-ONCOGENE IN BREAST CARCINOMAS OF YOUNG ARAB WOMEN

Fawziah M.A. Mohammed[1], Fatma Al-Yatama[1], Anwar Al-Banaw[1] and David T. Lincoln[2]

[1]Faculty of Allied Health Sciences, Kuwait University, Kuwait;
[2]Entity Systems, Independent Research Foundation, Chapel Hill, Australia

Breast cancer is characterized by a long history and marked heterogeneity in growth rates and clinical manifestations. In Europe and USA, occurence peaks between the ages of 60-65 years, with only 14% occurring below the age of 40 years. In contrast, in Gulf Arab women the mean age is 48.7 years and the peak occurrence of breast cancer is at the age of 45-50 years. A significant proportion of 26% of breast cancer occurs below the age of 30 years and the incidence is increasing. Only 14.1% of breast cancer in Arab women occurs above the age of 60 years. Breast cancer patients younger than 30 years have a worse prognosis than older patients. There are highly significant trends for the prevalence of poor prognostic features (Grade 3) histology, extensive intra-ductal component, lymphatic and vascular invasion to decrease with increasing age. In this study, we evaluated the expression of the HER-2/neu oncoprotein in breast cancer of young Arab women. The *HER-2/neu* gene codes for a putative transmembrane protein, similar in structure to the epidermal growth factor receptor (EGFR). A total of 122 patients were selected. The average size of the tumours was 5.6 cm, the majority of cases (76.2%) were invasive ductal carcinoma. The amplification/ overexpression of HER-2/neu from paraffin wax embedded breast tumour tissues was analyzed by immunohistochemistry using antiserum for presence of the HER-2/neu protein. A chemical detection system was applied to identify the antibody. A high degree of immunoreactivity indicated overexpression of this protein. From the 122 breast cancer cases investigated, 70 cases were positive, with different degrees of intensity ranging from weak (27 cases), moderate (23 cases) to strong (19 cases). The most intense HER-2/neu expression of the 70

total positive cases were found in ductal carcinomas (16 cases) and infiltrating ductal carcinomas (14 cases). Some cases (n=8) showed a correlation between the strongly positive HER-2/neu protein expression and the lymph node involvement. Only 6.8% of all cases investigated were Grade I well differentiated tumours, 41% were Grade II moderately differentiated and 22% were Grade III. In conclusion, the results of this study show that the degree of expression of HER-2/neu oncoprotein may prove to be an additional prognostic marker of breast cancer, particularly in young women. This applies in the histological diagnosis of ductal and infiltrating ductal carcinomas, followed by lobular, intraductal and infiltrating lobular carcinomas. The majority of these tumour etiologies were HER-2/neu positive.

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POTENTIAL ANTICANCER AGENTS FROM *LINUM GRANDIFLORUM* LEAVES

Magdy M.D. Mohammed[1], Ming Chen[2], Lin Zhai[2] and Nabaweya A. Ibrahim[1]

[1]Pharmacognosy Department, National Research Center, Dokki-12311, Cairo, Egypt;
[2]Department of Clinical Microbiology, University Hospital of Copenhagen (Rigshospitalet), Tagensvej 20, DK-2200 Copenhagen N, Denmark

The $CHCl_3$ and MeOH fractions of the leaves of *Linum grandiflorum* showed *in vitro* anticancer activity with IC_{50} against EL_4 (Murine Leukemia) cell line at 60 and 250 μg/ml respectively, using a standard high-flux anticancer-drug screening method. A bioassay guided fractionation was used to find out the active constituents, which resulted in the isolation of 10 compounds from the MeOH fraction classified as: 5 flavone glycosides (one novel compound known as luteolin 7-O-α-D-(6'''-E-feruloyl)glucopyranosyl (1→2)-β-D-glucopyranoside with IC_{50} against EL_4 at 125 μg/ml, together with, luteolin 7-O-glucosides, vicenin-1, vicenin-2 and vicenin-3) with IC_{50} against EL_4 at 100, 500, 500 and 500 μg/ml respectively, and 4 cyanogenic glycosides (one novel compound known as 2-[(3'-*iso*propoxy-O-β-D-glucopyranosyl)oxy]-2-methylbutanenitrile with IC_{50} against EL4 at 100 μg/ml, together with, linamarin, lotaustralin and neolinustatin) with IC_{50} against EL_4 at 100 μg/ml for each, and one alkyl glycoside (butan-2-*O*-β-D-glucopyranoside) with IC_{50} against EL_4 at 100 μg/ml. From the $CHCl_3$ fraction three lignans were isolated and identified as podophyllotoxin, deoxypodophylotoxin and 6-methoxypodophyllotoxin with IC_{50} against EL_4 at 90, 35 and 100 μg/ml respectively. The obtained results revealed the potent *in vitro* anticancer activity of *Linum grandiflorum* leaves and may suggest that the isolated active compounds could be used as anticancer agents.

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INHIBITION OF CARCINOEMBRYONIC ANTIGEN RELEASE FROM COLORECTAL CANCER CELLS TO PREVENT CRC-LIVER METASTASIS

Naghibalhossaini F. and P. Mokarram

Department of Biochemistry, Shiraz University of Medical Sciences, School of Medicine, Zand Street, Shiraz, Iran

Background: Elevated carcinoembryonic antigen (CEA) blood levels are found in a wide variety of epithelial neoplasms. CEA normal function(s) and its relevance to malignant transformation are not clear. Recent studies show that CEA might have an instrumental role in cancer progression and human colon cancer liver metastasis. The latter function has been suggested to be facilitated by soluble CEA through induction of cytokine production by Kupffer cells. We were the first group to provide evidence for CEA release from human colon cancer cells by an endogenous GPI-PLD enzyme. Previous studies have shown that purified GPI-PLD can be inhibited by micromolar concentrations of phosphatidic acid (PA) and lysophosphatidic acid (LPA). Therefore, blocking CEA release from cancer cells using GPI-PLD inhibitor might also prevent CRC-liver metastasis. *Methods:* A duplicate aliquot of LS180 cells was seeded into four-well plates (200,000 cells/well in 250 μl medium) and allowed to grow to sub-confluence. Then they were incubated in fresh DMEM medium with or without FBS containing GPI-PLD inhibitors, Didecanoyl phosphatidic acid (DP) and crude phosphatidic acid (CP). DP and CP were used at doses of 10-100 μM. The cells were incubated at 37˚C for 4-8 h in a humidified incubator. The media of controls were replenished with media without chemicals. The cell culture media from a duplicate set of wells was collected after every 2 h of incubation. The effect of inhibitors on the secretion of GPI-anchored proteins was determined by measuring CEA, and alkaline phosphatase (ALP) in cell culture supernatant by ELISA. The non-GPI-anchored CA19-9 tumor marker level in medium was also measured as a control. *Results:* LS-180 treatment with 20-100 μM concentrations of DP and CP reduced the release of CEA and ALP ($p<0.05$). No measurable alteration was observed at 10 μM PA concentration. A 19-57% decrease in CEA and ALP secretion was observed after 4-8 h continuous exposure of LS-180 cells to PA. *Conclusion:* GPI-anchored CEA and ALP, but not TM-anchored CA-19-9, release is inhibited efficiently by micromolar concentration of PA. Since secreted CEA acts as a signaling molecule that promotes liver metastasis, PA inhibition of CEA release from cancer cells may have therapeutic application to prevent CRC-liver metastasis.

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THERMODYNAMIC PROSPECTS TO INTERVENE CANCER PROGRESSION

Joseph Molnar[1], A. Zalatnai[2], B.S. Thornton[3], Elysia Thornton-Benko[4] and L. Amaral[5]

[1]Albert Szent-Györgyi Medical Center, Department of Medical Microbiology and Immunobiology, University of Szeged;
[2]1st Department of Pathology, Semmelweis University, Budapest, Hungary;
[3]Applied Mathematics, Faculty of Science UTS and School of Physics, University of Sydney;
[4]Key Centre for Health Technologies, UTS, Sydney, Australia;
[5]Unidade de Micobacterias, UMM, Instituto de Hygiene e Medicina Tropical, Lisboa, Portugal

During tumor growth there are many differences between the healthy tissue and growing tumor, including metabolic, structural and thermodynamic differences. The healthy state tends towards "entropy minimum", while the cancerous one tends towards "entropy maximum" by disrupting normal structures and functions invading host tissues based on different energy conservations and entropy productions. Indefinite growth of tumor cells can be inhibited by chemotherapy combined with resistance modifiers, but kinetic resistance of a solid tumor may needs an alternative solution involving thermodynamics.

The second law of thermodynamics does not exclude changes in the direction of components of entropy flow from tumor to the normal tissues under special conditions. Entropy productions due to various dissipation mechanisms based on temperature differences, chemical potential gradient, chemical affinity and exerted field as driving forces are promising tools to be calculated as potential targets for tumor demarcation. The relative importance of various terms of entropy production (migration of electric charges, the flow of matter, chemical reaction, heat transport) were compared as driving forces in tumor-host relationships through numerical estimation. Different entropy production rates between two kinds of cells determine the direction of entropy flow among corresponding tissues. The entropy flow carries the harmful information of a cancerous cell, propagating its toxic action to normal cells. Some inhibitors of quorum sensing-like signal transmission between tumor and normal cells can block the information flow.

External forces (ultrasound and electric fields) enhance entropy production of normal cells above cancer and consequently can reverse the direction of entropy flow. The entropy difference between exponential growth phase and Gompertzian phase is a possible target to eliminate tumor cells.

From topics in this review, new theories on the reduction of entropy production and negative entropy in-flow are now created in various biomedical fields, which may contribute to therapy.

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MDR INHIBITORY ACTIVITY OF NEW JATROPHANE DITERPENES FROM *EUPHORBIA ESULA* L.

E. Sulyok[1], A. Vasas[1], P. Forgo[1], J. Molnár[2] and J. Hohmann[1]

[1]Institute of Pharmacognosy, Faculty of Pharmacy, University of Szeged, H-6720 Szeged, Eötvös str. 6;
[2]Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, H-6720 Szeged, Dóm tér 10, Hungary

In continuing our search for biologically active new compounds from Euphorbiaceae species, four new jatrophane-type diterpene polyesters have been isolated along with three known components from the methanol extract of *Euphorbia esula*. The inhibition of efflux activity of the isolated compounds was determined by measuring the accumulation of the substrate analogue rhodamine in tumor cells. The isolation was carried out by VLC, CPC, preparative TLC and HPLC. The structure elucidation was performed by extensive spectroscopic analysis, including 1D and 2D NMR (^{1}H-^{1}H COSY, HSQC, and HMBC) experiments. The stereochemistry of the compounds was studied by NOESY measurements. Structural investigations demonstrated that compounds are penta-, hexa- and heptaesters of jatrophane polyols acylated with acetic, benzoic, isobutanoic and nicotinic acids. Four compounds are new natural products, two components were isolated earlier from *Euphorbia salicifolia* (1, 2) and one is identical with a diterpene obtained previously from *E. peplus* (3).

The effect of the isolated diterpenes on the reversal of multidrug resistance in *MDR-1* gene-transfected L5178 mouse lymphoma cells was determined. The assay was performed following extrusion of rhodamine 123 inside the cancer cells, by flow cytometry. Verapamil was used as a positive control. As a result of our investigations it can be stated that almost all of the tested compounds are able to increase drug accumulation and display a significant concentration dependent effect. These results provided further information for the characterisation of the structural requirements of jatrophane diterpenes as resistance modifiers.

1 Hohmann J *et al*: Tetrahedron *57*: 211-215, 2001.
2 Hohmann J *et al*: Tetrahedron Lett *42*: 6581-6584, 2001.
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PATHOLOGY BEYOND HISTOLOGY: PROGNOSTIC AND PREDICTIVE MARKERS IN GLIOMA TISSUE SAMPLES

Patrizia Morbini

Department of Pathology, University of Pavia, Pavia, Italy

Despite the huge amount of research done in the last decade on markers predictive of response to therapy and on target-specific drugs, only a few agents have so far been approved for target-based therapy of different malignancies, none of which involves the central nervous system, and a limited number of response predictors have been introduced into clinical practice.

On the verge of research for prognostic and predictive factors, pathologists' role has evolved in the last decade from she/he who provides a histological classification to a highly interactive player in the therapeutic decisions. The identification, by a constantly increasing range of molecular techniques, of predictive factors and potential targets in the tumor tissue is becoming the first step of the therapeutic approach for many tumor types and locations. Pathologists are also involved in ongoing research for new molecules and targets, in all fields of cancers research, including central nervous system tumor. A review of all recent data on the subject would require a lot of space, but MGMT and EGFR played so far the foremost role in the research of predictive and targetable pathways in GBL.

MGMT is a DNA repair protein that counteracts DNA damage induced by alkylating agents. In the majority of GBL, only one copy of the gene is left after chromosome 10 loss, and it can be epigenetically silenced by promoter hypermethylation. MGMT inactivation is responsible of GBL sensitivity to temozolomide, and its assessment has been proposed as a stratification criterion for therapy with alkylating agents.

EGFR is the transmembrane receptor of epidermal growth factor, and is the target of two FDA approved classes of inhibitors: monoclonal antibody cetuximab, targeting the extracellular receptor domain, approved for the treatment of intestinal adenocarcinomas, and small molecules gefitinib and erlotinib, which inhibit the intracellular ATP-binding domain of the receptor, and are used for the treatment of non small cell lung cancer.

GBL have been largely demonstrated to overexpress EGFR, mainly due to polisomy or gene amplification, as observed in bowel cancers, but also to mutations in EGFR gene, giving origin to the truncated, constitutively activated mutant EGFRvIII. Both EGFR overexpression and truncated protein variant are negative prognostic factors for GBL. Phase I and II clinical trials with small molecule EGFR inhibitors failed to show survival gain with respect to standard treatment with radiation and temozolomide. However, the presence of an intact downsteam signally pathway for EGFR seems to be necessary to confer inhibitor sensitivity to EGFR amplified or mutated tumors

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CONTROL OF ANTI-TUMOR CD8$^+$ T-CELL RESPONSES

David J. Morgan

Department of Cellular and Molecular Medicine, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK

Priming of naïve tumour-specific CD8$^+$ T-cells can occur either through direct interaction with metastatic tumours in the tumour draining lymph nodes (TDLN), or indirectly *via* dendritic cells (DC), cross-presenting tumour-derived antigens to naïve CD8$^+$ T-cells within the TDLN. However, the nature of CD8$^+$ T-cell response resulting from such interactions is determined by the overall level of T-cell activation. In the steady state, it was considered that high levels of T-cell activation tended to result in productive CTL responses to foreign antigens; whereas low levels of activation resulted in abortive responses leading to T-cell tolerance induction. However recent data, from our laboratory, has determined that the overall response following priming is, in fact, under dynamic control by a combination of several signals delivered through: the T-cell receptor TcR (signal 1), through receptors of costimulatory molecules and other accessory molecules (signal 2), as well as through several other molecular interactions, which collectively are referred to as signal 3. Critically, we have determined that altering the level of any one of these signals can result in a shift in the balance of the overall response between either abortive or productive activation. Based on these findings, we therefore suggest that tolerance induction and the formation of effective anti-tumour CTL responses actually represent discreet clinical readouts amongst a broad range of different CD8$^+$ T-cell responses.

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THE STUDY OF MANGANESE, CHROMIUM AND OXIDATION STATUS IN BLADDER CANCER

A. Movahedian, H. Mazdak, N. Mirkheshti, F. Yazdekhasti, E. Behzad, M. Shafian and Z. Shamszadeh

Department of Clinical Biochemistry, Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

It seems that chromium (Cr) and manganese (Mn), by the mechanism of affecting oxidative status in cells, can play an important role in cancer induction. We decided to study the serum concentration of Mn and Cr and malondialdehyde

(MDA) as a biomarker of lipid peroxidation and total antioxidant capacity (TAC) as an antioxidant marker, in patients with bladder cancer, as compared with healthy individuals.

This case–control study was conducted on 52 patients with bladder cancer and 58 healthy volunteers after being matched by their age, sex and smoking habits. Fasting samples were collected and serum concentration of Cr, Mn, MDA and TAC were determined by spectrophotometric methods and comparisons were made using the Student *t*-test.

MDA ($p<0.001$) and Cr concentration ($p<0.05$) were significantly increased in bladder cancer patients. There was a significant decrease in Mn ($p<0.001$) and TAC ($p<0.001$) of patients in comparison with healthy individuals.

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STANDARDIZED TISSUE AND BLOOD BANKING – PREANALYTICAL REQUIREMENTS FOR HIGH QUALITY GENE AND PROTEIN EXPRESSION PROFILES

T. Muley[1], C. Traschütz[1], H. Hoffmann[2], H. Dienemann[2], P.A. Schnabel[3] and M. Meister[1]

[1]Translational Research Unit;
[2]Dept. of Surgery, Thoraxklinik, University of Heidelberg;
[3]Institute of Pathology, University of Heidelberg, Germany

The identification of new prognostic factors, potential genes for targeting therapy, as well as genes that might cause resistance to chemotherapy are present goals in lung cancer research. The actual developments in molecular biology require high quality bio materials such as tumor tissue, serum, plasma or biopsy samples, since the quality of results directly depends on the quality of materials.

Currently, all necessary infrastructural requirements to achieve these goals have been installed and tested at our institution. Standard operation procedures have been implemented to provide the surgeons, pathologists and technicians with sampling protocols, ensuring high quality samples of tumour tissue and normal lung tissue pairs from each surgically treated patient. On average, 300–350 patients per year undergo surgical resection for primary lung cancer at our institution.

In brief, immediately after the tumour resection, representative pieces of tissue are macro dissected by an experienced pathologist from both the tumour and the distant normal lung tissue and cut into 16-20 pieces of 0.5 cm length. The pieces of tissue are distributed into sterile cryo tubes and immediately snap-frozen in liquid nitrogen. Long-term storage is performed at −80≥C. The time between resection and freezing is usually less than 30 min. In addition, in selected patients further tissue probes (pleural, lymph nodes, metastatic sites *etc*.) are withdrawn and prepared in an identical manner.

A piece of tissue is withdrawn from the freezer and cut into several 10 μm slices in a cryostat device before RNA, DNA or protein extraction and subsequent analysis. The first and last cryo-section of a series are Hematoxilin/Eosin-stained and analysed for tumor, stroma, necrosis and parenchyma content by two experienced investigators. Only tissues with greater than 50% viable tumor cell content enter RNA extraction and subsequent microarray analysis. RNA, DNA, and hybridisation products are analysed for integrity (RNA integrity numbers >8.0) and amount by capillary electrophoresis (Agilent Bioanalyzer) and micro photometry (Nano drop).

Basic patient data together with data of the freezing conditions are stored in a tissue bank data file. The inclusion of respective identification items (pseudonymized) allows us to combine tissue bank data to prospective data collected in the various data bases of our institution, i.e. tumour documentation files, documentation of surgery, laboratory data files, tumour biology/pathology data bases and postoperative follow-up files.

Taken together, standardized sample collection, sample preparation and data management is required to produce reliable high quality gene expression results which might in future guide new therapeutic approaches in lung cancer treatment.

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BIOMARKERS FOR ASSESSING POTENTIAL CARCINOGNEIC EFFECTS OF CHRONIC ARSENIC EXPOSURSE IN INNER MONGOLIA, CHINA

Judy L. Mumford

National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, USA

Arsenic is ubiquitous in the environment. Chronic arsenic exposure has been associated with carcinogenic, cardiovascular, neurological and diabetic effects in humans and has been of great public health concern worldwide. In 2001, the U.S. Environmental Protection Agency adopted an arsenic standard of 10 μg/l from 50 μg/l in drinking water. However, there are still great uncertainties on the health effects of arsenic at low doses. Due to the lack of appropriate animal models for extrapolation to human risk, research needs include a better understanding of the mechanisms of arsenic carcinogenesis and assessing health effects of arsenic in humans at low dose. The major modes of action (MOA) proposed for arsenic effects in humans are increasing oxidative stress, altering signal transduction pathways, inducing DNA and chromosomal damage, affecting DNA repair, promoting cell proliferation, and DNA methylation. The objectives of this research were to apply biomarkers to assess arsenic exposure and cancer risks and identify biomarkers useful for assessing arsenic effects in humans.

The residents of Ba Men in Inner Mongolia have been exposed to a wide range of arsenic concentrations *via* well water and showed adverse health effects. This population provides us with an opportunity to assess the relationships between arsenic exposure and health effects. Based on the proposed MOA, we selected biomarkers to assess potential carcinogenic effects of arsenic in the Inner Mongolia population. A total of 324 Ba Men residents, exposed to arsenic *via* well water ranging from non-detectable levels to 826 μg/l of arsenic participated in this study. Samples of well water, urine and toe nail were collected to assess arsenic exposure and investigate metabolic profiles in urine. Human buccal cells and blood samples were collected and the health effects investigated were arsenic-related dermal effects, DNA fragmentation, micronucleus frequency for chromosomal damage, and mRNA expression of the genes (*OGG1* and *ERCC1*) responsible for DNA repair of oxidative damage and human telomerase reverse transcriptase (*hTERT*) for cell proliferation.

The results showed significant associations between arsenic exposure and increase in DNA damage, micronucleus frequency and gene expression of *OGG1* and *ERCC1* and *hTERT*. This paper will report the results of these studies and provide some insights into arsenic MOA and human dose-response data.
This is an abstract of a presentation and does not necessarily reflect U.S. EPA policy.

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CHEMOPREVENTIVE EFFECTS OF DIALLYL TRISULFIDE DERIVED FROM GARLIC: FOCUS ON MOLECULAR MECHANISMS

Hye-Kyung Na and Young-Joon Surh

National Research Laboratory of Molecular Carcinogenesis and Chemoprevention, College of Pharmacy, Seoul National University, Seoul 151-742, South Korea

Multiple lines of compelling evidence from epidemiological and laboratory studies support an inverse association between consumption of garlic and the risk of cancer. Chemopreventive effects of garlic have been attributed to its oil-soluble sulfur ingredients, such as diallyl sulfide, diallyl disulfide, and diallyl trisulfide (DATS), but their underlying molecular mechanisms remain largely unresolved. In the present study, we observed that DATS inhibited the growth of human breast carcinoma (MCF-7) cells to a greater extent than did the other allyl sulfides as determined by the MTT assay. DATS also induced apoptosis in MCF-7 cells, which was mediated through accumulation of reactive oxygen species with subsequent activation of JNK that catalyzes phosphorylation of Bcl-2. In another experiment, DATS prevented tumor formation in a mouse xenograft model. Aberrant upregulation of COX-2 has been frequently observed in several types of cancer cells and is considered as a molecular target for cancer chemoprevention. Topically applied DATS inhibited the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced COX-2 expression in dorsal skin of female ICR mice. DATS inhibited the DNA binding activity of AP-1 which is one of key transcription factors regulating the inflammation and expression of COX-2. DATS inhibited TPA-induced phosphorylation of Akt and JNK, which are major MAPKs regulating AP-1. A pharmacologic Akt inhibitor LY294002 and a JNK inhibitor SP600125 abrogated the TPA-induced COX-2 expression, suggesting that suppression of COX-2 expression by DATS in TPA stimulated mouse skin is mediated by blocking the PI3K-Akt and JNK signaling. Topical application of DATS protected against mouse skin carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene plus TPA. In addition, DATS strongly inhibited DNA binding activity of NF-κB compared with other allyl sulfides in human mammary epithelial (MCF10A) cells treated with TPA. DATS inhibited the transcriptional activity of NF-κB, phosphorylation of IκBα, and activity of IKKβ. Inhibition of NF-κB DNA binding activity and IKKβ activity by DATS were blunted by addition of the antioxidant N-acetyl-L-cysteine (NAC) and the reducing agent dithiothreitol.

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CLINICAL SIGNIFICANCE OF WNT-INDUCED SECRETED PROTEIN-1 (WISP-1/CCN4) IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

Youhei Nagai[1], Shinji Ishikawa[2], Yoshihumi Baba[3], Ryuichi Karashima[1], Nobutaka Sato[1], Yu Imamura[1], Yukiharu Hiyoshi[1], Naoya Yoshida[1], Eiichirou Toyama[1], Naoko Hayashi[1], Masayuki Watanabe[1] and Hideo Baba[1]

[1]Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University 1-1-1 Honjyo, Kumamoto City, Kumamoto 860-8556;
[2]Department of Surgery, Kumamoto Regional Hospital, Kumamoto 860-0811, Japan;
[3]Dana-Farber Cancer Institute, 35 Binney Street JF208E Boston, MA 02115, USA

Background: Wnt-induced secreted protein-1 (WISP-1/CCN4), a member of the CCN family, has been studied in several cancers. However, the correlation of WISP-1 expression with clinical features of esophageal squamous cell carcinoma remains unknown. *Patients and Methods:* The expression of WISP-1 was analyzed by immunohistochemistry on paraffin-embedded tissues from 105 cases of esophageal squamous cell carcinoma. The effect of WISP-1 on esophageal carcinoma cell growth was examined by cell proliferation assay using WISP-1 transfectants. *Results: In vitro*, WISP-1-transfected esophageal cells showed significantly increased proliferation compared to their parent cells. Immunohistochemical analysis showed that WISP-1 positive cases were closely associated

with tumor size, lymph node metastasis, stage and worse prognosis. *Conclusion:* WISP-1 may play a important role in tumor growth and development of esophageal squamous cell carcinoma.

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THE HAMSTER BUCCAL POUCH CARCINOGENESIS MODEL AS A PARADIGM FOR ORAL ONCOGENESIS AND CHEMOPREVENTION

S. Nagini

Department of Biochemistry and Biotechnology Annamalai University, Annamalainagar-608 002, Tamil Nadu, India

Oral squamous cell carcinoma (OSCC), a common malignancy worldwide, is an important contributor to the overall international cancer burden. Squamous cell carcinomas (SCCs) induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) in the HBP reiterate many of the features observed in human OSCCs. The major risk factors associated with human oral cancer such as tobacco, betel quid and alcohol promote HBP carcinogenesis. SCCs induced by DMBA in the cheek pouch of Syrian hamsters are morphologically and histologically similar to human OSCC. Like human oral carcinogenesis, HBP carcinogenesis is a multistep process that involves sequential progression from hyperplasia to invasive carcinoma through varying degrees of dysplasia. In addition, HBP tumours express several biochemical and molecular markers that are also expressed in human OSCC. Multiple signaling pathways are dysfunctional in both human and hamster OSCCs. In particular, cell proliferation, apoptosis and angiogenesis are intricately interlinked in malignant transformation of the HBP mucosa by DMBA. The HBP carcinogenesis model is the best-known animal system for intervention by chemopreventive agents because of easy accessibility for examination, and follow-up of lesions. A number of synthetic and natural products have been documented to exhibit chemopreventive efficacy in the HBP model. Chemoprevention studies in the HBP model can serve as a crucial link in the potential efficacy assessment of candidate agents for oral cancer prevention and therapy.

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ORAL CANCER AND ITS RELATIONSHIPS WITH SALIVA, CIGARETTE SMOKING AND FREE RADICALS – PREVIOUS FINDINGS AND FUTURE TSPO-RELATED DIRECTIONS

R. Nagler

Department of Oral and Maxillofacial Surgery, Oral Biochemistry Laboratory and Salivary Clinic, Rambam Medical Center and Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Oropharyngeal (OP) cancer, which is usually squamous cell carcinoma, is the most common head and neck malignancy and accounts for 2-4% of all new cancer cases. It is primarily induced by exposure to tobacco. The paradigm of cigarette smoke (CS)-induced OP cancer pathogenesis is based on the assumption that a constant direct attack by various CS carcinogens causes widespread accumulating cellular and DNA aberrations in the OP mucosal cells, in turn eventually resulting in malignant transformation. However, there is never direct contact between CS and the OP mucosa. Saliva, bathing the mucosa from the oral cavity to the larynx, always intervenes, and CS must first interact with saliva before it reaches the mucosa.

The current study investigated the role of saliva in the pathogenesis of OP cancer. A synergistic effect of CS and saliva on oral cancer cells was demonstrated. This synergism is based on the reaction between redox active metals in saliva and low reactive free radicals in CS, which results in production of highly active hydroxyl free radicals. Thus when exposed to CS, salivary behavior is reversed and the saliva loses its antioxidant capacity and becomes a potent pro-oxidant milieu. The devastating role of CS-borne aldehydes was demonstrated as well. Based on these results and on our recent reports demonstrating that CS destroys various salivary components, including protective ones such as peroxidase, the most important salivary antioxidant enzyme, a comprehensive view of the pivotal role of saliva in the pathogenesis of CS induced OP cancer is suggested.

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EFFECT OF mTOR INHIBITOR ON HUMAN OSTEOSARCOMA CELL LINES

Osamu Nakamura, Kanji Sasaki, Toshiaki Hitora, Yoji Kawaguchi and Tetuji Yamamoto

Kagawa University, Orthopedic Surgery, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa-ken, Japan 761-0701

Introduction: The mammalian Target Of Rapamycin (mTOR) is the downstream of the PI3K/Akt signaling pathway. It plays an important role in cell proliferation and apoptosis. Rapamycin is commonly used as an immunosuppressor, however, it also has an inhibitory effect on mTOR. Several authors have reported the antitumor effects of Rapamycin on many human malignancies. The inhibition of mTOR is involved in the pathway-mediated transcription and translation, leading to cell cycle arrest, antiangiogenesis, and apoptosis. We examined the expression of mTOR-gene, and the inhibitory effects of Temsirolimus, a selective mTOR inhibitor, in human osteosarcoma cell lines. *Materials and Methods: Cell lines and reagents*. Three human osteosarcoma cell lines (KTHOS, MG63 and KHOS) were

used. KTHOS was previously established in our laboratory. All cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich). The cell lines were routinely maintained at 37˚C in a humidified 5% CO_2 atmosphere. Temsirolimus, an inhibitor of mTOR was purchased from Wyeth Pharmaceuticals. *mRNA expression of mTOR*. Total RNAs were eluted by selective binding to a silica-gel-based membrane using an RNeasy Mini Kit® (QIAGEN Inc., Valencia, CA). Reverse transcription of RNA into cDNA was performed by using Reverse Transcription System (Promega, Madison, WI). mRNA of mTOR expression were examined by reverse transcription (RT-) PCR. After PCR amplification, 8 μl aliquots of the PCR products were electrophoresed in a 2% agarose gel, followed by ethidium bromide dye. *The inhibitory effect of mTOR inhibitor.* Cell proliferation was determined using the MTS assay (CellTiter 96® Aqueous One Solution Cell Proliferation Assay; Promega, Madison, WI). Cells were seeded in 96-well cell culture plates. After 24 hours (h), the medium was refreshed with 1% FBS containing Temsirolimus at various concentrations. After 24 and 48h, the medium was removed and washed with phosphate buffered saline, then refreshed with fresh medium containing MTS reagent. The optical density was measured at 490 nm using an automatic microplate reader after 2 h of further incubation. The percent viability of each well was calculated. At least three independent cultures were performed for each study. *Results:* The mRNA of mTOR was strongly expressed in all cell lines. Temsirolimus inhibited the cell proliferation of all 3 human osteosarcoma cell lines in a dose- and time-dependent manner. *Conclusion:* These results suggest that through the mTOR dependent intracellular signaling pathway, mTOR- targeting agents become an important chemotherapeutic strategy for human osteosarcomas.

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EVALUATION OF IMATINIB MESYLATE (STI571) EFFECTS ON GLIOBLASTOMA CELL PROLIFERATION AND MIGRATION

E. Ranza[1], G. Mazzini[2], A. Facoetti[3,4] and R. Nano[1,4]

[1]Department of Animal Biology, University of Pavia;
[2]IGM-CNR, Istochem. and Cytometry Section, Pavia;
[3]Department of Nuclear and Theoretical Physics, University of Pavia;
[4]INFN Pavia, Italy

Glioblastoma is a highly malignant brain tumour with limited therapeutic options, a high recurrence rate and mortality. Therefore the search of improved treatment is urgently needed.

The fundamental insight into signal transduction generated during the past decade is being translated into a new generation of specific inhibitors. These drugs can be very selective in action and may provide opportunities for glioblastoma treatment. One of these new drugs is imatinib mesylate (STI571), a small molecule, inhibitor of PDGF receptor, implicated in astrocytoma carcinogenesis. In this study, two human glioblastoma cell lines (T98G and A172) were analysed for their sensitivity to treatment with imatinib mesylate (kindly provided by Dr E. Alessandrino). In particular, we have focused our attention on the analysis of the effects on cell proliferation and migration after imatinib treatment. Our results show that STI571 is able to induce growth arrest in the G_0/G_1 phase of the cell cycle at all concentrations tested already 24 hours after treatment and apoptosis after 48 hours at 20-30 μM. Moreover, we have seen that the imatinib treatment determines an evident decrease of migrating cells. These data suggest that at low concentrations STI571 could act as a cytostatic agent but at high concentration it behaves mainly as a cytotoxic agent.

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EFFECT OF IMATINIB MESYLATE (STI571) IN COMBINATION WITH γ-IRRADIATION ON ASTROCYTOMA CELL SURVIVAL

E. Ranza[1], A. Bertolotti[2,3], A. Facoetti[2,3], F. Pasi[1,3], A. Ottolenghi[2,3] and R. Nano[1,3]

[1]Departments of Animal Biology, and [2]Nuclear and Theoretical Physics, University of Pavia;
[3]INFN Pavia, Italy

Imatinib mesylate is a small molecule inhibitor of the c-Abl, c-Kit, platelet-derived growth factor (PDGF) receptor tyrosine kinases that is approved for the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors and is under investigation for several other malignant tumors. Malignant astrocytomas are brain tumours with an extremely poor prognosis that are usually treated with surgery and/or radiotherapy. Recently, we have found that imatinib is able to induce a growth arrest in G_0/G_1 phase of cell cycle in astrocytoma cells, suggesting a possible cytostatic effect of this new drug. In this study, we focused our attention on the potential role of imatinib (kindly provided by Dr E. Alessandrino) in radiotherapy of astrocytoma by determining the response of human malignant astrocytoma cell lines (MOG-G-UVW and T98G) to combination treatment with imatinib and γ-rays. The effect of irradiation with and without imatinib pre-treatment on cell survival was tested with clonogenic assay.

Our results demonstrate that PDGFR inhibition by imatinib only partially decrease the viability of glioblastoma

cells, but in combination with γ-irradiation there is a significant increase of this effect. In particular, we have found that low-concentrations of imatinib are able to specifically enhance the radiosensitivity of astrocytoma cells. These results suggest that imatinib may have clinical utility as a radiosensitizer in the treatment of human astrocytoma.

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IMMUNOBIOLOGICAL ASPECTS OF GLIOMA AND IMPLICATIONS FOR IMMUNOTHERAPY

Rosanna Nano[1] and Enrica Capelli[2]

Departments of [1]Animal Biology and [2]Genetic and Microbiology, University of Pavia, Pavia, Italy

Today various findings indicate that a multifactor strategy is the best strategy for treating cancer. Immunotherapy could be a role in this fight as evidence suggests that the immune system has a role in the control of malignant growth and metastasis. Whether the immune system can target tumours has been debated for nearly a century. Various investigations, clinical trials and animal based studies have produced important scientific knowledge for the development of a really effective immunotherapy Actually immunotherapy could be a promising approach for the development of integrative therapies for cancer. Cancer immunotherapy has progressed towards clinical applications and various approaches have been developed, such as adoptive transfer of tumour specific cytotoxic T lymphocytes and the administration of tumour-associated antigens-component vaccine, genetically modified tumour cell-based vaccine, TAA-coding DNA vaccine on TAA-delivered dendritic cell (DC)-based vaccine. Compared to a drug delivery system, which delivers the optimal amount of drug to the target site and subsequently elicits its effect *via* a chemical compound or by biologic macromolecules such as plasmids, a cell based system which uses smart cells existing in the body is a potentially, not yet completely studied, approach. It requires a precise knowledge of the immune network that involves cytokines, chemokines, effector function of leukocytes subsets such as NK and T cytotoxic cells

Many scientific centres in the world studied the immunological aspects of tumour-host interactions and experienced therapeutic trials. The data obtained from these studies are now an important source for further strategies

In some cancers as glioblastoma, a chemo- and radio-resistant tumour, immune recruitment could be a basis to develop new approaches. It is necessary to focus studies on the tumour-host relationship between and on cytokines that regulate these relationships in the micro environment.

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DEVELOPMENT OF ANTICANCER NANOPARTICLES TO ENHANCE SELECTIVITY, APOPTOSIS, IMAGING, AND THERAPY

Arutselvan Natarajan, Gary Mirick, Gerald L. DeNardo and Sally J. DeNardo

Radiodiagnosis and Therapy, University of California Davis Medical Center, 1508. Alhambra Blvd, Sacramento, CA - 95816, USA

Objectives: Dextran and PEG-coated iron oxide nanoparticles (NPs), and gold nanoparticles (GNPs) have been widely used to target cancer cells when suitably modified with molecular-targeting ligands and/or apoptosis-inducing agents. Anticancer monoclonal antibodies, scFvs, microRNAs, peptides, cytotoxic molecules, and small high affinity ligands (SHAL) have been conjugated to NPs and GNPs to increase valency and circulation time to enhance the cancer-targeting effect and specificity for imaging and therapy. We have developed a series of cancer-targeting multifunctional nanoparticles (MNPs) to enhance the ligand concentration in tumor cells for imaging, therapy, and to induce apoptosis. MNPs were characterized by PAGE, transmission electron microscopy (TEM), cellulose acetate electrophoresis (CAE), inductively coupled plasma mass spectroscopy (ICPMS) and live cell-binding assays. Pharmacokinetics in athymic mice bearing human breast cancer (HBT 3477) and lymphoma (Raji) xenografts were studied. *Results:* Four tumor targeting MNPs were prepared: MAb-NP (20 nm), di-scFv-NP (20 nm) and SHAL-GNP (10 nm) and miRNA-GNP (10 nm). The first three MNPs have been used for animal studies. They were >90% monomeric by PAGE and CAE. The amount of anticancer agents conjugated to each NP was 3-10 μg/mg of NPs and 0.05-1 μg of SHAL or miRNA/mg of GNPs. Tumor uptake of (% ID/g ± SD) for each MAb-NP, di-scFv-NP and SHAL-GNP at 48 h was 13±0.12 (20 nm), 5±0.08 (20 nm) and 5±0.3 (10 nm), respectively. The miRNA-GNP was effectively transfected into cells to kill cancer cells *in vitro*. *Conclusion:* Four anticancer MNPs were generated, characterized and evaluated for their cancer-targeting effect. PK and WBAR demonstrated 20 nm NPs and 10 nm GNPs targeted tumor *in vivo*. Apoptosis-inducing mRNA and small high-affinity ligand (SHAL) were conjugated to10 nm GNPs. MAb and di-scFvs linked to NPs and SHAL-GNPs, targeted tumors 5-13% at tumor in mice. MiRNA-conjugated GNPs enhanced the apoptotic effect in cancer cells by >30% compared to miRNA alone. Development of imaging and therapy strategies for these novel anticancer MNPs are ongoing.

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EXPRESSION, MUTATION, AND FUNCTION OF MET RECEPTOR TYROSINE KINASE AND

DOWNSTREAM TARGETS IN NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS AND LUNG CANCER

Viswanathan Natarajan and Ravi Salgia

Department of Medicine, The University of Chicago, Chicago, IL-60637, USA

Lung cancer is a devastating illness and usually occurs as non-small cell lung cancer (NSCLC, incidence of 85%) and sometimes as small cell lung cancer (SCLC, incidence of 15%). Even with the best therapies with chemotherapy, radiation therapy, and surgery, we have not made considerable strides in overall survival. The prognosis for NSCLC is 16% over 5 years and 6% for SCLC. Most recently, novel targeted therapies have come to fruition in lung cancer. As an example, the epidermal growth factor receptor (EGFR) has been targeted with small molecule inhibitors such as gefitinib or erlotinib, or with an antibody against EGFR such as cetuximab. Even with this novel target, the response rate is still only 15%. Thus, we had to find other molecular targets for lung cancer. The MET receptor tyrosine kinase has been shown to be important in cell proliferation, growth, angiogenesis, invasion, and metastasis for a number of tumors. We have shown that MET and its activated form (p-MET against the juxtamembrane domain–pY1003) are overexpressed in lung cancer tumor tissues (as compared to adjacent normal lung tissues). The ligand for MET is the hepatocyte growth factor (HGF), and can be either expressed in the stroma or the tumor tissues. Expression of MET in tumor tissue microarrays was quantified in a 4-tier score system as negative (0), weak (1+), moderate (2+), or strong staining. In lung cancer, forty percent (16/40) of lung cancer tissues overexpressed MET. In the tissue microarray, 20% (1/40) had no expression (0), whereas 20% (8/40) had 1+, 35% (14/40) had 2+, and 5% (2/40) had 3+ MET expression. In lung cancer, 73% expressed phosphor-MET (1+, 14/40; 2+, 13/40; 3+, 2/40) while 27% (11/40) did not. HGF was also expressed in lung cancer (47% (19/40) had 1+, 45% (18/40 had 2+, and 5% (2/40) had 3+). We have also analyzed mutations/single nucleotide polymorphisms (SNPs) of *MET* in lung cancer, and have identified novel mutations in the semaphorin (SEMA) ligand-binding domain and the juxtamembrane (JM) domains and not the tyrosine kinase domain. In determining the JM domain function, we show that there was activation of the downstream target paxillin (focal adhesion protein). MET activation also led to downstream signaling with signaling through the focal adhesion proteins (such as paxillin), PKC pathway, and AKT pathway. This was true both for normal human bronchial epithelial cells and lung cancer cells. We are currently investigating the therapeutic inhibition of MET in lung cancer, and show that small molecule inhibitors such as SU11274 and PHA665752 lead to a decrease in cell growth as well as a decrease in angiogenesis for the mouse model. In summary, the MET pathway is important and will be a crucial therapeutic target in lung cancer.

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TARGETED CANCER THERAPY WITH CATIONIC LIPOSOMES, CURRENT ACHIEVEMENTS *VERSUS* CLINICAL NEEDS AND FUTURE OPTIONS

Kurt W. Naujoks

Pharma Research Consulting Comm. V., Square de Meeus 37 - 4th Floor, 1000 Brussels, Belgium

All cancer therapies developed so far suffer more or less from two main problems: i) induction of severe side-effects and ii) induction of tolerance to the respected drug.

Multiple attempts to cope with these problems resulted in the development of target-specific therapeutics. Most successful are monoclonal antibodies (mab) and kinase inhibitors. Both drug concepts helped to improve cancer treatments, but benefits fade after 3 to 12 months due rising drug tolerance. In addition, it turned out that there are indeed side-effects to be coped with.

Another target associated with new hope was the tumor vasculature as a non-tumor tissue, but with vital functions for the tumor. Several approaches using small molecules failed due to the complex biology; monoclonal antibodies (*e.g.* Bevazicumab - rhuMAb-VEGF) however made it successfully to the clinics. But here also, side-effects do require specific attention.

A totally different approach used a simple physical-chemical interaction to target the neovasculature of tumours. Based on an observation that cationic liposomes exhibit a preferential binding to activated or proliferating endothelial cells, a new system of target-specific delivery of drugs and contrast agents was developed. Tumor-specific binding was demonstrated in various preclinical models and in a diagnostic Phase I study in bladder carcinoma patients. Subsequently therapeutic studies in various indications demonstrated therapeutic effects at doses which were well tolerated. Surprisingly, it could be shown that prolonged treatment improved therapeutic benefits rather than showing induction of tolerance. In a Phase II study in pancreatic cancer, a new drug, EndoTAG 1 (formerly LipoPac) demonstrated a prolonged survival compared to that with best standard of treatment. However this experimental drug so far fails to meet the standard requirement for successful drugs as the treatment requires infusions of 4 litres or more over several hours. In addition, preparation of the infusion requires specific instructions of the respective hospital pharmacy to dissolve the

lyophilised product. Pharmaceutically, the production process is not optimized either.

This presentation will outline the approach taken to develop such a promising drug from a prototype formulation to a drug matching the day to day requirements of a cancer care unit and the new technology to meet pharmaceutical production requirements.

Progress achieved to date: Drug volume reduced to 15%; the encapsulation efficacy of therapeutic drugs in cationic liposomes could be greatly enhanced using different compositions and lipids.

Infusion reactions and overall tolerability could be significantly improved by adding an outer layer to the liposomes; thereby creating a new kind of nanoparticle which has no tendency to form aggregates when injected into the blood.

A new way to stabilise these nanoparticles allows for both better scaling-up of production and better reconstitution of the resulting powder as a drug for infusion.

Preclinical results with this new nanoparticle will be presented.

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QUANTITATIVE STUDIES OF THE CELLULAR IMMUNE RESPONSE IN CERVICAL CANCER

B.S. Nedergaard[1], M. Ladekarl[2], H.F. Thomsen[3], J.R. Nyengaard[4] and K. Nielsen[5]

Departments of [1]Oncology and [5]Pathology, Aalborg Hospital, Aarhus University Hospital;
Departments of [2]Oncology and [3]Clinical Epidemiology, Aarhus University Hospital;
[4]Stereology and Electron Microscopy Laboratory and MIND Center, University of Aarhus, Denmark

Using stereology we presented a method to obtain basic biological data on the *in situ* cellular immune response towards cancer. We estimated the density and frequency of immune cells of 10 different phenotypes in cone biopsies from patients with stage I cervical squamous cell carcinoma (APMIS *115*: 1321-1330, 2007). Using this method, we performed a study to investigate differences in the primary *in situ* cellular immune response between patients with and without relapse of stage IB cervical squamous cell carcinoma. We found significantly lower densities of CD3+, CD4+ and CD8+ cells (both intra- and peritumoral) in tissue from patients who had relapse (Gynecologic Oncology 108 (2008) 106-111). To validate the results, a cohort study including 102 patients treated for cervical squamous cell carcinoma stage IB and IIA between 1990 and 2000 at Aalborg Hospital, Denmark was carried out. The *in situ* cellular immune response was investigated with respect to densities of T-cells (CD3+), T helper/regulatory cells (CD4+) and cytotoxic T-cells (CD8+) in intra- and peritumoral tissue. We found an increase in the density of both CD3+ and CD8+ cells to decrease the hazard ratio for relapse of disease. The decrease in hazard ratio was highly significant for both intra- and peritumoral cells. The largest decrease in hazard ratio was found for peritumoral CD3+ cells and it was 0.27 when increasing the cell density from 795 to 2043 cells/mm2 (25 to 75 percentile). According to this study, a low density of particularly peritumoral CD3+ cells is associated with increased risk of relapse in squamous cell cervical cancer (BJC *97*: 1135-1138, 2007).

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EXPRESSION OF THE TMPRSS2:ERG FUSION GENE AND PROSTATE CANCER PROGRESSION

Gabriella Nesi[1], Lorella Bonaccorsi[2], Milena Paglierani[1], Csilla Krausz[2], Marco Carini[3], Yuqiang Fang[4], Suresh C. Jhanwar[4], Gianni Forti[2], Elisabetta Baldi[2] and Lucio Luzzatto[5]

Departments of [1]Human Pathology and Oncology, [2]Clinical Physiopathology, Unit of Andrology, [3]Critical Care Medicine and Surgery, Unit of Urology, University of Florence, Italy;
[4]Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, USA;
[5]Istituto Toscano Tumori, Florence, Italy

The TMPRSS2:ERG fusion is a common recurrent chromosomal aberration in prostate cancer. Some data suggest that ERG expression is higher in less aggressive prostate carcinomas, while others show an association between TMPRSS2:ERG fusion gene and more aggressive disease. We assessed the TMPRSS2:ERG fusion status in tumour samples from 84 patients undergoing radical prostatectomy from 1998 to 2000. Sixty patients had surgery alone (group A), while 24 patients received androgen ablation therapy for 3 months before surgery (group B). The presence of the TMPRSS2:ERG gene fusion product was demonstrated by reverse transcription polymerase chain reaction (PCR) in 84% of group A patients and in 54% of group B patients (p=0.01). The levels of TMPRSS2:ERG and ERG, measured by means of real-time quantitative PCR, did not show significant association with clinicopathological characteristics of the tumours, except for a negative correlation of ERG overexpression with Gleason score (R=0.457; p=0.0001), observed in group A alone. The lower proportion of group B patients harbouring TMPRSS2:ERG fusion suggests that androgen ablation inhibits the expression of TMPRSS2:ERG, underscoring the key role of androgen-mediated transcription control of the gene fusion. Differently to group A, group B patients expressing the fusion gene experienced earlier biochemical (PSA) recurrence (p=0.007). In this specimen set, we observed that tumours in which androgen ablation prior to surgery fails to suppress expression of the fusion gene have a greater risk of

recurrence. Further studies in larger cohorts of tissue are under way to confirm these findings.

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DENDRITIC CELL-BASED IMMUNOTHERAPY: INFLUENCE OF THE MICROENVIRONMENT ON THE IMMUNE RESPONSE

Thomas Neßelhut, Dagmar Marx, Constanze Matthes, Dirk Lorenzen and J. Neßelhut

Institute for Tumour Therapy, Hinterstraße 53, 37115 Duderstadt;
Department of Immunology, University of Göttingen, Germany

Especially in advanced stages of cancer, common therapy strategies show insufficient results accompanied by a reduced quality of life due partly to strong side-effects. Today, one of the most promising innovative therapy approaches in treatment of various types of human cancer is a specific immunotherapy with dendritic cells. Several groups have shown that immunotherapy with monocyte-derived dendritic cells (MoDC) loaded either with tumor-cell–lysate, known tumor-specific peptides, or with specific RNA or DNA can induce a clinical antitumor response in patients with various types of cancer accompanied by fewer side-effects and good tolerability. Nevertheless, most of the treated patients fail to respond to the therapy. The ineffective clinical antitumor response to a dendritic cell-based therapy may be partly due to an inflammatory tumor microenvironment. Several investigations showed increasing evidence of a strong association between chronic infection, inflammation and cancer development as well as in tumor progression. Many of the same inflammatory mediators that are secreted by wounds are found in the tumor microenvironment. It is known that also tumors themselves can change the immunological balance into an inflammatory microenvironment leading not only to promotion of tumor growth but also to inhibition of an efficient immune response and thus resulting in an unsuccessful immunotherapy based on dendritic cells.

Besides several cytokines which promote an immune suppressive microenvironment, such as for example IL-10, regulatory T-cells (T-reg) play an important role in regulation of an immune response. An increase of regulatory T-cells is often found in cancer patients and may limit the antigen-specific immune response. To reduce these T-reg cells to a normal range, a metronomic chemotherapy should be considered as pretreatment prior to an immune therapy with dendritic cells, which may enhance the antigen-specific CD-8-T-cell response. Moreover, an anti-inflammatory treatment should be accompanied by a noninflammatory microenvironment, which can promote DC activation and can enhance tumor immunity as well as the clinical antitumor response. An efficient induction of a clinical antitumor response requires, beside a change of the immune suppressive tumor-associated microenvironment, a polarization of MoDC in a TH1 direction. However, less IL-12 and high IL-10 production by the MoDC favoring a TH2 immune response rather than a TH1 response is often found in cancer patients. Culture conditions with supplement of certain TLR ligands can signal the presence of infection (“danger signal”) and may overcome the possible defective MoDC of cancer patients. Using culture conditions with sequential supplementation of a synthetic lipopeptide and LPS, we are able to change an IL-12/IL-10 production profile <1 to an IL12/IL-10 production profile >1, which favours a TH-1 response. Furthermore we show that activation of the Toll-like receptor 3 by adding (poly I:C), to the cultures as well as NDV can lead to DC, which are able to produce IL-12 p70 at a higher level than IL-10. The IL-12/1L10 ratio seems to be correlated to the clinical response. Thus, addition of TLR agonists can effectively improve the maturation status as well as the TH1-polarisation of MoDC. Taken together, a successful dendritic cell based immunotherapy requires more information on the cancer characteristics to define the appropriate patient.

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RADIONUCLIDE BASED TUMOUR TARGETING OF CD44-POTENTIAL APPLICATIONS IN HEAD AND NECK CANCER

Marika Nestor

Unit of Otolaryngology and Head and Neck Surgery, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

CD44 is one of the most complex molecules in all biology. It is a multistructural and multifunctional cell surface molecule involved in cell-cell and cell-matrix interactions and signal transduction. Alternative splicing can theoretically generate hundreds of CD44 isoforms, and posttranslational modifications further enrich the structural variability of the molecule. CD44 is essential to multiple biological functions of normal cells, but is also crucial to many tumour cell activities. Malignant cells often express unique patterns of CD44 isoforms, and CD44 is one of the most common markers used for isolation of cancer stem-like cells. This makes it a highly interesting candidate for selective cancer targeting.

We have assessed the CD44 isoform CD44v6 for radionuclide based tumour targeting of head and neck cancer. Radionuclide conjugates were created using the anti-CD44v6 chimeric monoclonal antibody (cMAb) U36 as a targeting molecule. The conjugates ^{211}At-cMAb U36 and ^{131}I-cMAb U36 were preclinically evaluated for therapeutic use in squamous cell carcinoma (SCC) cells. A combination of the

anti EGFR-antibody cetuximab and ^{211}At-cMAb U36 was also evaluated to further improve the therapeutic efficacy. Results proved the astatinated conjugate to be most efficient, demonstrating a specific and dose-dependent cytotoxicity. Cetuximab influenced the therapeutic efficacy, but did not mediate the same reaction in all cell lines, demonstrating the wide variability of SCCs. Finally, the conjugates ^{124}I-cMab U36 and ^{111}In-cMab U36 were evaluated for diagnostic use in tumour bearing mice. Results were promising, with favourable biodistributions and good visualizations in micro-PET and planar gamma camera, respectively.

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NEW UNDERSTANDINGS OF CANCER AND PROLIFERATION FROM NANOGENOMICS AND NANOPROTEOMICS

Claudio Nicolini

Nanoworld Institute, University of Genova, Corso Europa 30, 16132 Genoa, Italy and Fondazione ELBA, Piazza SS. Apostoli 66, 00100 Rome, Italy

A new approach for cancer research is emerging from nanogenomics (1) and nanoproteomics (2) as interplay of bioinformatics, mass spectrometry and biomolecular microarrays proceeds to a previously unforeseeable level. We summarize here its major features with a few key applications, such as the cell cycle progression of human T lymphocytes (3), the reverse transformation of CHO-K1 hamster fibroblasts (4), human organ transplant (5) and cancer (2, 4).

While investigation of the whole CHO-K1 proteome during c-CAMP reverse transformation appear problematic by 1D or 2D SDS PAGE, HPLC and MALDI-TOF after a subcellular fractionation in four distinct fractions (4), nanogenomics (1) and nanoproteomics (2) allow the study of an immense number of genes and/or proteins with only one experiment and can therefore draw unique picture of the whole genome and proteome. The huge volume of data arising from pangenomic and panproteomic microarray experiments may often increase experimental complications and difficulties in the analysis. Despite the power of combined *ab-initio* analysis and experimental analysis (1) genomics does however suffer many pitfalls and only functional proteomics, called nucleic acid programable protein array detecting the human proteins produced by genes directly in a mammalian milieu during the assay (2), represents the long-term answer to the basic molecular understanding and to the clinical control of human cancer, mainly in the Label-Free approach jointly developed within the framework of an ongoing cooperation between Harvard Institute of Proteomics and Genova NanoWorld Institute.

These microarrays are produced by printing the proteins of interest on the array using methods designed to maintain the integrity and activity of the protein, allowing hundreds to thousands of target proteins to be simultaneously screened for function using a wide range of gene collections. The focus of function-based microarrays is to study the biochemical properties and activities of the target proteins printed on the array. Function-based microarrays can be used to examine protein interactions with other proteins, nucleic acids, lipids, small molecules and other biomolecules. NAPPA and DNASER microarrays are introduced here to identify the key proteins and key genes in the G_0/G_1, G_1/S, S/G_2, G_2/M and M/G_0 transitions induced by PHA in resting lymphocytes. Kidney transplantation is another medical problem successfully approached with nanogenomics, permitting a microarray- and bioinformatic-based identification of key genes controlling tolerance and rejection of human kidney transplant respectively. The whole proteome is instead approached with mass spectrometry and with Label-Free technologies (AFM, Nanogravimetry and Anodic Porous Alumina electrochemistry). The extremely high density and aspect ratio of APA represent the future challenges of Nanoproteomics (6).

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CALCIUM AS MODULATOR OF VITAMIN D MEDIATED ANTI-TUMOUR POTENTIAL IN THE COLON

Thomas Nittke, Heide S. Cross and Enikö Kállay

Department of Pathophysiology, Medical University Vienna, Austria

Epidemiological and experimental studies emphasise the importance of adequate vitamin D and calcium intake for prevention of colorectal cancer. The active vitamin D metabolite $1,25\text{-}(OH)_2D_3$ plays an important role in

differentiation and apoptosis of several cells, having a strong anti-proliferative effect. In the colon 1,25-$(OH)_2D_3$ bound VDR regulates also the expression of key detoxifying enzymes, contributing to the elimination of mutagenic compounds. In early colon tumuorigenesis, expression of the enzyme catalysing synthesis of the active vitamin D_3, 25 hydroxyvitamin D 1α hydroxylase (CYP27B1) is increased whereas that of the catabolising hydroxylase CYP24A1 is very low. In advanced tumours it is vice versa the expression of CYP24A1 is highly elevated. Calcium might support local, colonic 1,25-$(OH)_2D_3$ accumulation by regulation of CYP27B1 and CYP24A1 expression and thereby affect tumour progression. In this context we analysed the expression patterns of Cyp27b1, Cyp24a1 and, the detoxifying enzyme Cyp3a11 in the colon mucosa of mice fed either low or high calcium diet. In parallel we measured 1,25-$(OH)_2D_3$ levels and apoptosis with respect to functional relevance of the obtained mRNA profile. Mice fed with low calcium exhibited high Cyp24a1 mRNA levels in the right colon. At the same site, expression of Cyp27b1 was also upregulated, but only in females. This was accompanied by an increased level of 1,25-$(OH)_2D_3$ and by increased apoptotic activity. The same gender preference was observed for the Cyp3a11 expression in the right colon: in females Cyp3a11 was upregulated by low dietary calcium.

Our data suggest that in healthy colon mucosa the adverse effects of low nutritional calcium could be counteracted by enhanced synthesis of 1,25-$(OH)_2D_3$. This might be responsible for increased apoptosis and possible improvement of detoxification. Those defence mechanisms against tumour development appear to be restricted to the right colon and are more effective in females.

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INFLAMMATION AND INFLAMMATORY PROCESSES IN TUMOR ANGIOGENESIS AND METASTASIS, MECHANISMS AND PREVENTION

Adriana Albini[1], Girieca Lorusso[1,2], Agostina Ventura[3], Lorenzo Mortara[2], Michael B. Sporn[4], Antonio Sica[5] and Douglas M. Noonan[2]

[1]IRCCS Multimedica, Milan;
[2]Università degli Studi dell'Insubria, Varese;
[3]Centro Biotecnologia Avanzate, Genova, Italy;
[4]Dartmouth Medical School, Hanover, New Hampshire, USA;
[5]Istituto Clinico Humanitas, Milan, Italy

It is now clear that components of the innate immune system are one of the driving forces of tumor angiogenesis. This suggests that these cells may be effective targets for therapies aimed at curbing tumor progression, however their potential has yet to be fully explored. For example, the angiogenesis inhibitor angiostatin, identified by functional assays *in vivo*, appears to preferentially target immune cells. Angiostatin is unable to exert angiogenesis inhibition in the presence of function blocking antibodies to IL-12 or in mice with gene-targeted deletions of either the IL-12-specific receptor IL-12Rβ2, or the IL-12 p40 subunit. *In vitro*, treatment of human macrophages with angiostatin alone induced mRNA significantly to elevate synthesis of the two IL-12 subunits, as determined by real-time PCR. These data indicate that macrophages are a primary target of angiostatin and place the immune system at a central fulcrum in the regulation of angiogenesis *in vivo*. Furthermore, they indicate that endogenous angiogenesis inhibitors appear to function by targeting immune cells rather than endothelial cells, which indicates these cells are attractive targets for combination anti-angiogenic therapies.

Our studies on the mechanisms of cancer chemoprevention compounds, indicate that a key and common activity is a block of angiogenesis, a concept known as angioprevention. We have also found that angioprevention compounds, including the flavonoids epigallocatechin 3 gallate and xanthohumol, often inhibit the NF-κB pathway, a central molecular hub for inflammation. Since synthetic oleanane triterpenoids have been observed to inhibit the NF-κB pathway, we investigated the anti-angiogenic potential of a series of synthetic triterpenoids, including CDDO, CDDO-Im and the CDDO-methyl ester (CDDO-Me, methyl 2-cyano-3,12-dioxoolean-1,9-dien-28-oate). While all inhibited angiogenesis, CDDO-Me showed remarkable potency while being well tolerated *in vivo*, effective at doses as low as 0.003 mg/kg of body weight. In addition to the NF-κB axis, CDDO-Me and related triterpenoids also inhibit STAT and TGFβ signaling. The particularly potent anti-angiogenic activity seen *in vivo* suggests that CDDO-Me may be interacting with an entire network of molecular and cellular targets, rather than at a single molecular locus or in a single cell type.

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THE EFFECT OF ANDROSTENEDIONE ON PROLIFERATION OF OVARIAN EPITHELIAL CANCER CELLS

M. Nourbakhsh[1], M. Zahraei[1], A. Golestani[1], MH. Modarressi[2] and F. Karami-Tehrani[3]

Departments of [1]Clinical Biochemistry and [2]Medical Genetics, School of Medicine, Medical Sciences/University of Tehran, Tehran;
[3]Department of Clinical Biochemistry, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran

Introduction: Ovarian cancer is the leading cause of gynecological cancer mortality, and more than 85% of this malignancy arises from the ovarian surface epithelium. The

etiology of epithelial ovarian cancer and the mechanism of its progression are poorly understood, but there is increasing evidence that reproductive hormones regulate the growth of ovarian cancer cells. Epidemiological studies have revealed that high levels of androgens are implicated in increasing the risk of ovarian cancer. The aim of this study was to determine the proliferation of ovarian cancer cells in response to androstenedione which is the main androgen of the ovary. *Materials and Methods:* Ovarian carcinoma cells (OVCAR3) were cultured in RPMI-1640 containing 10% FBS in 24- and 96-well plates and allowed to adhere for 24 hours. Then the cells were switched to medium supplemented with dextran-coated charcoal-treated FBS in the presence or absence of 10^{-5}-10^{-9} M 4-Androstene-3,17-dione. Cellular proliferation was assessed by means of MTT assay and cell counts with hemocytometer and trypan blue. *Results:* The proliferation of ovarian cancer cells was significantly increased by androstenedione in a time-dependent manner, and all applied concentrations of androstenedione resulted in significantly higher numbers of cells than the untreated control. *Conclusion:* We have demonstrated that androgens can cause an increase in proliferation of ovarian cancer cells.

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NBS1 GENE MUTATIONS AS A CANCER RISK FACTOR

Jerzy Nowak, Maria Mosor, Iwona Ziółkowska, Agnieszka Dzikiewicz-Krawczyk and Danuta Januszkiewicz

Institute of Human Genetics Polish Academy of Sciences, 60-479 Poznan, Strzeszynska 32, Poland

MRE11, RAD50 and NBS1 (MRN) complex is involved in DNA repair and cell cycle checking signaling. NBS1 being a part of MRN complex plays an important role in genome stabilization. Molecular variants of *NBS1* gene may therefore constitute a cancer risk factor. Homozygous mutation 657del5 of the *NBS1* gene is responsible for the majority of Nijmegen breakage syndrome. Several studies have focused on searching for an association between *NBS1* gene mutations and cancer incidence. Heterozygous carriers of the *NBS1* 657del5 mutation have been shown to have an increased risk for breast cancer, melanoma, colon and rectum cancer. Other studies have found no association between *NBS1* gene mutations Hodgkins's or non-Hodgkin's lymphomas. The aim of the study was to analyze the frequency of a panel mutations of NBS1 gene by screening all 16 exons of this gene along with polymorphisms examination. DNA was isolated from peripheral blood of 135 children with acute lymphoblastic leukemia, 270 women with breast cancer, 176 patients with larynx cancer, 93 with second primary tumors of head and neck, 131 with colorectal carcinoma and 1274 healthy individuals. I171V mutation of *NBS1* gene was the most frequent and has been found in 23 patients compared to only 8 in healthy individuals. Other mutations of the *NBS1* gene have been observed in lower frequencies. Genotyping data from the six polymorphic loci in *NBS1* gene, were used to impute haplotypes. Two of the evaluated haplotypes were associated with significantly increased leukaemia risk (p=0.0038 and p<0.0001). Our results suggest that some specific haplotypes of the *NBS1* gene may be associated with malignancies. Since DNA was also isolated from non-malignant cells, all mutations found in cancer patients appeared to be of germinal origin. It can be concluded that I171V mutation of *NBS1* gene is associated with predisposition to malignancies and *NBS1* allele I171V may be a general cancer susceptibility gene of low or middle risk.

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THE _IN VIVO_ EFFECT OF FENUGREEK ON METABOLISM OF ARACHIDONIC ACID IN AN ANIMAL EXPERIMENT

Ghodratollah Nowrasteh, Timea Varjas, Ferenc Budán, Krisztina Amrein, Gabor Horváth and Istvan Ember

Institute of Public Health, Faculty of Medicine, University of Pécs, 12 Szigeti st., H-7643 Pécs, Hungary

The main bioactive compounds of *Trigonella foenum graecum* (fenugreek) seeds are protodioscin, trigoneoside, diosgenin and yamogenin. These have been found to have anticarcinogenic potency in different settings, such as inhibition of cell proliferation and inhibition of prostaglandin synthesis *e.g.* metabolic pathway with lipoxygenase (ALOX) and cyclooxygenase (COX) enzymes. The seed extracts of fenugreek can also be of help in chemoprevention.

In our experimental set up we examined the anti-inflammatory effect of fenugreek on fatty acid metabolizing enzymes (ALOXs and COXs) in AKR/J H-2^k mice exposed to carcinogen dimethylbenz[α]anthracene (DMBA). We also determined the gene expression pattern of enzymes involved in arachidonic acid metabolism based on detecting the mRNA expression in various tissues (lung, liver, spleen and kidney) in four groups of mice. Two groups were fed with a normal diet (control) and two of them consumed fenugreek-containing diet, and each group received DMBA treatment. All groups were autopsied on the 7th day, 24 hours post carcinogenic exposure, and mRNA from target organs was isolated. Gene expression did not change in the tissues of mice on the fenugreek diet in comparison with mice on a normal diet in control group.

DMBA exposure increased the expression of *ALOX12* mRNA in the liver, lung and spleen of the mice consuming

fenugreek, but in the kidney, fenugreek diet suppressed gene expression to a normal level. *ALOX15* gene expression was increased by carcinogen in all organs examined, but in the liver, lung and kidney fenugreek consumption reduced the elevated gene expression to a normal level. *ALOX5* gene expression was increased by 2.5 to 4-fold in all organs due to carcinogen exposure; the inhibitory effect of fenugreek was detected only in the liver and kidney. The expression of COX1 enzyme (exception of spleen) increased in response to DMBA exposure in all examined organs and the fenugreek diet suppressed gene expression independently of carcinogen exposure. The increased expression of COX2 in response to DMBA was suppressed by fenugreek in all of the four tissues examined.

The arachidonic acid metabolism pathway is one of the possible targets of specific molecular prevention therapy. According to the literature, prostate, lung, and other cancer cell lines express ALOX5, with a metabolism that leads to the formation of a variety of metabolically active products with different roles in carcinogenesis. Fenugreek can be a supplementary factor in chemoprevention.

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PROTEASES AND COLLAGEN-DERIVED ANGIOGENESIS INHIBITORS IN THE TUMOR MICROENVIRONMENT

Pia Nyberg and Tuula Salo

Department of Diagnostics and Oral Medicine, Institute of Dentistry PO Box 5281, FIN-90014 University of Oulu, Finland

It has become clearly evident that tumor growth does not just depend on the carcinoma cells, but instead various cell types, such as fibroblasts and endothelial cells, as well as extracellular matrix (ECM) components affect the outcome. The ECM is both a backbone and a barrier for cells as well as a storage place for biologically active molecules. Therefore proteases cleaving ECM component are crucially important. Proteases can be either protective or destructive in cancer. We have studied tumor-associated trypsin-2 that is a protease that correlates with the aggressiveness of cancer. Trypsin-2 increases metastasis formation by activating other downstream proteases as well as by disturbing tight junction molecules. The turnover of ECM liberates many biologically active cryptic molecules that possess anti-angiogenic activity. Arresten and endostatin are examples of such endogenous angiogenesis inhibitors that are derived from collagen IV and XVIII, respectively. They inhibit angiogenesis and tumor growth, but their mechanisms are not completely known. It is important to note that even though the collagen-derived endogenous angiogenesis inhibitors are molecules of about the same size, come from similar parent molecules and have sequence homologies, they function *via* distinct mechanisms, bind different cell surface receptors, and affect angiogenesis and the tumor microenvironment in distinct ways. We have shown that arresten affects endothelial cell proliferation, migration and apoptosis *via* integrin $\alpha1\beta1$. Here we aimed to elucidate how arresten and endostatin affect the behaviour of aggressive oral carcinoma cells and normal and cancer-associated fibroblasts. We have previously shown that endostatin inhibits the activity of matrix metalloprotease-9 (MMP-9). Here we show that arresten has quite the opposite effect: it increases gelatinase (MMP-9 and -2) production by oral carcinoma cells and normal and carcinoma-associated fibroblasts. Furthermore, arresten increases oral carcinoma cell migration, whereas endostatin inhibits carcinoma cell migration. However, in an organotypic model, arresten inhibits the invasion of carcinoma cells. In conclusion, our data demonstrates how different the effects of collagen-derived angiogenesis inhibitors can be in the tumor microenvironment despite of the similar effects on endothelial cells. When designing treatment strategies for cancer, the effects on all the components of the tumor microenvironment need to be elucidated. The organotypic carcinoma models are a great tool to achieve this goal.

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RADIONUCLIDE DETECTION OF NEUROENDOCRINE TUMORS USING ^{111}In PENTETREOTIDE

Vladimir Obradović, Vera Artiko, Nebojša Petrović, Dragana Šobić, Milan Petakov, Ivana Božić, Tatjana Isailović and Svetozar Damjanović

Institute for Nuclear Medicine , Institute for Endocrinology and Metabolic Diseases, Clinical Center of Serbia, Belgrade, Serbia

Aim: The aim of the study is the detection of primary and metastatic neuroendocrine tumors using ^{111}In pentetreotide (OctreoScan), which is a long-acting analog of somatostatin. *Patients and Methods:* A total of 30 patients was investigated. Scintigraphy of the whole body, (and tomography 360˚/6˚ if necessary) was performed 4h - 48 h after *i.v.* administration of 111MBq ^{111}In pentetreotide. *Results:* In the group with 12 neuroendocrine carcinomas of unknown origin, there were 10 true positive (TP) findings (6 with liver metastases, one with liver, lung, and bone metastases, one with liver and mediastinal lymph node metastases and 2 with liver and retroperitoneal lymph node metastases), while 2 were false negative (FN) (poorly differentiated carcinoma with retroperitoneal metastases). In 6 patients scintigraphy influenced further patient management. From the group of 12 pancreatic

neuroendocrine tumors, in 8 neuroendocrine pancreatic carcinomas, there were 6 TP (4 with liver metastases) and 2 FN (poorly differentiated). In 2 patients with pancreatic gastrinomas findings were TP, while in 2 patients with insulinoma one was TP and in the other TN. In 6 patients scintigraphy influenced further patient management while in 4 only contributed. From the group of 6 neuroendocrine lung tumors there were 4 TP (4 patients with bronchial carcinoid, two with liver metastases and the other two with liver, lung and bone metastases), in 1 patient with atypical lung carcinoid after surgery, findings were TN, while in one with neuroendocrine lung tumor (ACTH secreting) it was FN (small mediastinal tumor. In 2 patients scintigraphy influenced further patient management while in 2 only contributed. Because of the high uptake of radiopharmaceutical, and widespread metastases, six patients were indicated for radionuclide therapy with ^{90}Y-DOTA TATE, and three of them received it. *Conclusion:* Preliminary results show that scintigraphy of neuroendocrine tumors is a useful method in diagnosis, staging and follow up of the patients suspected to have neuroendocrine tumors in the lungs. It is also helpful in the appropriate choice of therapy, including radionuclides.

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PARASPORIN, A NEW ANTICANCER PROTEIN GROUP FROM *BACILLUS THURINGIENSIS*

Michio Ohba[1], Eiichi Mizuki[2] and Akiko Uemori[1,3]

[1]Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka;
[2]Biotechnology and Food Research Institute, Fukuoka Industrial Technology Center, Kurume;
[3]Fresenius Medical Care Japan K.K., Tokyo, Japan

Bacillus thuringiensis was first isolated in 1901 from Japan as a silkworm pathogen. It produces proteinaceous parasporal inclusions during sporulation. The inclusions often exhibit strong and specific insecticidal activity. This makes *B. thuringiensis* an environmentally safe agent for control of medically and agriculturally important insect pests. Earlier studies, however, have also demonstrated that *B. thuringiensis* strains with non-insecticidal inclusions are more widely distributed than insecticidal ones. Mizuki *et al.* (1999) first reported that strong cytocidal activities against human cancer cells are often associated with the non-insecticidal inclusion proteins. Subsequently, we obtained a cytocidal protein gene, leading to the establishment of a new functional protein group, parasporin (Mizuki *et al.*, 2000). Currently, parasporin is defined as "parasporal proteins produced by *B. thuringiensis* and related bacteria that are non-hemolytic but capable of preferentially killing cancer cells."

At present, parasporin (PS) is classified by its amino acid identity into four subgroups, PS1 to PS4, consisting of a total of 13 proteins. PS1 and PS3 have structural features of three-domain insecticidal Cry proteins. PS2 and PS4 are small-type parasporins with no structural similarity to the known *Bacillus* parasporal proteins. PS1 is synthesized as an 81-kDa precursor protein that is proteolytically converted into an active heterodimer of 15 and 56-kDa polypeptides. It exhibits specific toxicity against uterine cancer cells (HeLa) but is not active on normal uterus smooth muscle cells (UtSMC) and normal T-cells. PS1 induces extracellular Ca^{2+} influx into cytoplasm and subsequent apoptotic cell death of susceptible cells. The PS2 precursor of 37 kDa is changed into a 30-kDa active form through proteolysis. The protein is not toxic to normal hepatocytes (HC) but is highly toxic to hepatocyte cancer cells (HepG2) and leukemic T-cells (Jurkat). PS2 molecules target lipid rafts, oligomerize and form pores in the rafts of cell membrane of the susceptible cancer cells. The precursor of PS3, an 88-kDa protein, is converted into a 64-kDa active protein by protease. PS3 has a structural similarity to the insecticidal three-domain Cry proteins. It exhibits preferential toxicities against limited numbers cancer cells, including myeloid leukemic cells (HL60). The mode of action of PS3 is less characterized, while poreformation seems to be key action of this protein. PS4 is a small-type parasporin, a 31-kDa precursor and a 27-kDa active form. The protein exerts cytotoxicity on colon cancer cells (CaCO-2) and leukemic T-cells (MOLT-4), but not on normal T-cells. It is very likely that PS4 molecules, like PS2, oligomerize and form pores in cancer cell membranes. Elucidation of the origin of the specificity associated with PSs is currently under way, and the identification of specific receptors, if any, may lead to the use of these unique proteins for medical purposes.

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IRINOTECAN IN THE TREATMENT OF COLORECTAL CARCINOMA: LIGHTS AND SHADOWS

Cristiano Oliva, Paola Bergnolo and Alessandro Comandone

Department of Medical Oncology, Presidio Sanitario Gradenigo, Torino, Italy

In the last five years the introduction of the so called "biological drugs" has moved the attention of the researchers on this new kind of compounds. This is probably due to new mechanisms of action, new fields of interest, new toxicities, new drug combinations.

Irinotecan is an S-phase-specific derivative of camptothecin which interferes with DNA replication and cell division by inhibiting topoisomerase-I. It has demonstrated antitumor activity against metastatic colorectal cancer. Irinotecan is a versatile drug, it can be administered at different doses every

three weeks, every two weeks and following weekly schedules. It has a well known spectrum of toxicities, that are not cumulative and that can be mostly predicted and treated. Adverse events most frequently recorded were neutropenia, acute cholinergic syndrome, fatigue, nausea and vomiting, delayed diarrhea and alopecia. It has been approved by FDA for the first line treatment of colorectal cancer since 1997 and remains stil nowadays one of the three fundamental cytotoxic drugs for this disease. The mechanism of action and single agent efficacy of IRI, combined with the apparent absence of any cross resistance with FU provided the rationale for combining IRI with FU and Folinic Acid as the first line therapy for metastatic colorectal cancer.

Irinotecan has thus been tested in combination with the infusional Fluorouracil (FU)/Folinic Acid (FA) schedule at different dosages and different intervals of administration. Vanhoefer *et al*. tested IRI in a weekly setting in combination with the AIO German FU/FA schedule in a phase I study. They reached the maximum tolerated dose (MTD) of weekly IRI at 100 mg/m^2 in association and recommended further studies with a lower dose (80 mg/m^2). Ducreux associated IRI with the FU/FA de Gramont schedule and defined the recommended bimonthly dose of IRI at 180 mg/m^2. In a large phase III study by Douillard *et al*. this association was statistically superior to the de Gramont regimen alone in terms of response rate (34.8% *vs*. 21.9%), time to progression (TTP) (6.7 *vs*. 4.4 months) and survival (17.4 *vs*. 14.1 months). From that time the fact that Irinotecan combination with FU/FA significantly increases response rates, TTP and survival in patients with metastatic colorectal cancer has become always more evident.

The other effective drugs in Colorectal cancer are FU and Oxaliplatin. Following the results of the well known "Mosaic" study, the Oxaliplatin's use has progressively moved toward the adjuvant setting. Moreover Oxaliplatin is a "one bullet gun" and its use is strongly conditioned by its cumulative neurotoxicity. Its use is restricted to the adjuvant setting or first line treatment. However, the improvements in the results of the treatment of advanced disease lead patients to receive also three or more different lines of chemotherapy in this setting. Therefore, patients with advanced colorectal cancer will receive therapies mostly based on the combination 5FU (or oral fluoropyrimidines) plus Irinotecan, variously administered.

In our phase II study we choose to combine the weekly schedule of IRI (80 mg/m^2) with a 28 days protracted venous infusion of FU. This resulted in an active combination with a mild toxicity profile. 52 pts were accrued in the trial. We recorded 5 Complete Responses (9.6%) and 15 Partial Responses (28.8%) with an overall response rate of 38.5% (95% C.I. 25% to 52%). Moreover, we had 17 patients with Stable Disease (32.7%) for a disease control rate of 71.2% (95% C.I. 58% to 84%). We detected a median PFS of 8.2 months (95% C.I. 6.1 to 10.1 months) and an OS of 16.3 months (range 4-58+ months, 95% C.I. 14.8 to 17.9 months).

In our daily Clinical Practice we usually prescribe the Wi-Fi regimen also to patients already treated with one line of chemotherapy not containing Irinotecan (mostly FOLFOX or Oxaliplatin plus Capecitabine). Data from this second line slighty differ from the first line results both for response rate and overall survival. Interesting data regarding similar symptoms and free survival are available.

Thus resection was judged not feasible before therapy. Thirteen patients (25%) underwent surgical resection for their metastatic disease after 4 to 6 courses of chemotherapy. Although in only 10 cases resection was considered macroscopically radical. After the resection of their metastatic disease patients received at least two courses of Wi-Fi with a IV stage adjuvant purpose. In this setting a phase III randomized trial by M. Ychou *et al*. has recently been reported. The Authors randomised pts with completely resected liver metastases, to receive 12 courses of FU/FA according to DeGramont schedule versus 12 courses of FOLFIRI. Primary endpoint was Disease Free Survival. No differences were observed in the DFS between the two arms, adjusted or not for important prognostic factors. The role of Irinotecan in the adjuvant setting has been established in a lot of large randomized clinica trials. Neither the Saltz's trial (CALGB C89803) nor the ACCORD-2 and the PETAC-3 trials were able to find significant differences between FU/FA based chemotherapy and FU/FA plus Irinotecan regimens.

In our study the toxicities recorded were mild and a median number of 5 courses of chemotherapy was administered. However, 11 patients had to interrupt the treatment because of toxicity. 4 of them had problems related to central venous catheter. In three cases the patients developed a deep venous brachial or jugular thrombosis and one patient had rupture of the venous catheter. In this latter case, the subsequent procedures to recover the broken part induced the patient to decide to stop the treatment. In our opinion, the problems with the venous catheters are a field in which the substitution of the protracted infusion of FU with oral fluoropyrimidine could result in a better cost-effectiveness ratio. However, the recent data from the BICC-C study, a randomized trial comparing the standard FOLFIRI regimen to irinotecan and bolus FU/FA (IFL regimen) and to an association of IRI plus capecitabine, reports the control arm to be superior in terms of response rate, PFS and OS with a better toxicity profile. These findings were confirmed in the EORTC study 40015 in which C. H. Köhne *et al*. compared the classical FOLFIRI regimen versus a Capecitabine plus Irinotecan schedule called CAPIRI. Both regimens were randomly assigned to a therapy with celecoxib or placebo. The trial was prematurely closed because of an unexpected number of deaths unrelated to disease progression but it was possible to establish that both TTP and OS were shorter for CAPIRI than for FOLFIRI. These findings can reaffirm the importance of the infusional FU-based therapies even in the era of oral fluoropyrimidines.

In our opinion the Wi-Fi combination is an active schedule that well matches with the weekly administration of Cetuximab. A phase II study of the combination Wi-Fi plus Cetuximab has already started in our Department. On the basis of recent advances in the use of Cetuximab we decided to reserve this treatment to patients with wild-type K-RAS disease.

A lot of trials established that Capecitabine is at least active as infusional FU. On the contrary why Irinotecan seems to work better when combined with FU than when associated with Capecitabine? Irinotecan works well in the advanced setting but it does not seem to be equally active in the adjuvant setting, either stage II-III or stage IV.

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VARIOUS POSSIBLE METHODS FOR INDUCING UROTHELIAL TUMOURS IN RATS AND MICE USING CHEMICAL COMPOUNDS

P.A. Oliveira

CECAV, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal

The bladder is one of the most common sites for the appearance of cancer in the urinary tract. Bladder tumours are manifestations of a multifocal disease whose natural history has not yet been completely elucidated. Animal models provide a system that can enhance our understanding of basic biology. To induce the development of urothelial tumours, it is essential to select correctly the model that is most analogous to the clinical settings so that observations can be readily transferred to clinical studies for validation. Over the past few decades, research efforts have focused on the development of rodent models that permit the reproducible induction of bladder cancer with minimal or no induction of tumours in other organs. Several chemicals, such as *N*-butyl-*N*-(4-hydroxybutil)-nitrosamine, *N*-methyl-*N*-nitrosourea and *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide, have proven particularly effective at this, and when administered *via* the appropriate route, at the appropriate dosage and in the appropriate strain of animal, all produce a high incidence of bladder tumours. By monitoring responses to chemical carcinogens using experimental models, it has been possible to identify many of the mechanisms through which tumours develop and to evaluate new therapeutic strategies. In our oral communication, the various possible methods for inducing urothelial tumours in rats and mice using chemical compounds, the spectrum of histological lesions observed in each model and the results of my recent research will be presented.

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CHEMO-PREVENTION OF: PROSTATE CANCER: THE RATIONALE BEHIND DESIGN OF PILOT STUDIES

Tim Oliver, Attila Lorincz and Jack Cuzick

Wolfson Institute of Preventive Medicine, Queen Mary University London, Charterhouse Square, London EC1M 6BQ, UK

12 years ago one of us (TO) first proposed the hypothesis that prostate cancer was caused by a life-time of "sub-clinical" prostatitis. The article also speculated on the basis of the first results from use of intermittent hormone therapy that short-term (1-3 months) androgen blockade given at age 45 (to the one third of the population known from post mortem studies to have latent prostate cancer at that stage) could be a very effective form of chemo-prevention. The hypothesis was based on a considerable paucity of hard facts and mainly rested on three borderline significant aetiological similarities in common between prostate and cervix cancer – *i.e.*, risk of malignancy was increased by 1) early onset of sexual activity, 2) reduced by circumcision, and 3) increased in Afro-Caribbean's. Further support came from the observation that 1 in 5 patients with advanced metastatic prostate cancer treated by intermittent androgen blockade (IAB) survived for prolonged periods (5 or more years) without further need for treatment. Subsequently, studies on a cohort of predominantly 25-35 year old South African Gold miners demonstrated a correlation between both early initiation of sexual activity and evidence of chlamydial infection with increased PSA levels and provided additional support for the hypothesis that repeated episodes of minor sexually transmitted diseases (STD) might cause lasting damage to the prostate. The subsequent demonstration by others that at the age of 45 those with a PSA above the median level had a 3.75 higher risk of death from prostate cancer provided further evidence of the value of PSA as a screening tool for prostate cancer detection. However, it also raised questions whether this is because PSA level also reflects the degree of persistent sub-clinical prostatitis and that is associated with cancer development rather than a direct measure of existing prostate cancer.

Today, with five reviews in major journals, the concept of chronic inflammation of the prostate as a major cause of prostate cancer has become mainstream. The failure to associate a single major pathogen with cause, unlike helicobacter gastritis in stomach cancer, has so far resulted in few therapeutic endeavours and led to the conclusion that the association is multi-factorial including non-specific infections, autoimmunity and dietary/environmentally acquired chemical toxins. The demonstration in a rat model that intra-urethral *E. coli* leads to the development of proliferative inflammatory atrophy and also Prostatic Intra-epithelial Neoplasia (PIN) has demonstrated how a non-specific infection can become a major driving force in the clonal development of this disease.

This presentation will review supporting data prior to focusing on the major paradox of medical therapy of

prostate cancer, ie that PSA testing leads to over diagnosis and thus active monitoring to select those in need of radical treatment is acceptable. However, a recent meta-analysis of immediate vs deferred androgen blockade randomised trials has shown survival advantage though an unacceptable burden of side effects for immediate treatment. The past year has seen the publication of the first results from The *I*nternational *S*tudy of *I*AB for *Ca*rcinoma of the *P*rostate (*ISICaP*) Group that has undertaken an individual patient data meta-analysis of IAB studies This has provided the first solid evidence that short course (3 months) IAB offers a potentially safe alternative to continuous anti-androgen therapy. More importantly from the point of view of the chemo-preventative potential of short course treatment, this analysis has also demonstrated that a higher proportion of earlier cases remain progression free for 3 or more years off treatment. In addition good progress has been reported in development of urine and semen based tests for diagnosing prostate cancer.

Based on these observsations and recognition of the high cancer risk of PSA positive biopsy negative individuals possible chemo-prevention protocols for such patients combining short term anti-androgens, anti-Cox 2s and high dose Vitamin D are under consideration. The rationale behind these proposals and their design will be reviewed

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MULTIPLE MYELOMA – CHEMOKINE NETWORK: CCL27/ CTACK – A NEW PLAYER IN MYELOMA PROGRESSION

Angelika Olivier[1], Michael Unger[1], Eva Maizner[1], Daniel Neureiter[2], Richard Greil[1,3] and Karin Jöhrer[1]

[1]Tyrolean Cancer Research Institute, Innrain 66, A 6020 Innsbruck;
[2]Institute of Pathology at the Private Medical University Hospital Salzburg, Müllner Hauptstrasse 48, A 5020 Salzburg;
[3]3rd Medical Department with Hematology, Medical Oncology, Hemostaseology, Rheumatology and Infectious Disease at the Private Medical University Hospital Salzburg, 5020 Salzburg, Austria

Multiple myeloma is a still incurable B cell neoplasm characterized by the monoclonal expansion of plasma cells in the bone marrow. The physical interaction of multiple myeloma cells with compartments of the bone marrow microenvironment has a crucial role in the pathogenesis of the disease. In detail, promotion of tumor growth and survival, angiogenesis or the homing of the malignant clone are sustained by the bone marrow milieu. Chemokines have currently been shown to be major players in shaping the tumor microenvironment of multiple myeloma cells.

Investigating the bone marrow supernatants of multiple myeloma patients we found the chemokine CCL27/ CTACK distinctly produced. This chemokine, which has so far only been correlated with skin associated tumors, was significantly upregulated in myeloma bone marrow compared to MGUS patient samples. Additionally its expression could be correlated with the stage of the disease. Immunohistochemical data revealed that myeloma plasma cells as well as endothelial cells are the major source for the production of this protein.

Due to the known implications of the chemokine network in multiple myeloma, we started to reveal the possible role of this chemokine in tumor biology. What we found so far was the induction of some tumor promoting qualities. In detail the chemokine CCL27 induced the migratory capacity of myeloma cell lines, augments their susceptibility to IL-6 -a major growth factor for myeloma tumor cells- and enhances proliferation.

An alternative way of myeloma cells to progress is the modulation of immune cell subsets in order to favour the tumor. With regard to the supporting role of dendritic cells in myeloma disease, and since they are the most potent antigen presenting cells inducing T cell responses, we focused on this cell subset. Monocyte derived dendritic cells – differentiated and matured in the presence of CCL27- exhibited a reduced capacity to activate T cells and this correlated with reduced cytokine production on the T cell side. Further these dendritic cells exhibited an impairment in migrative behaviour but at the same time induced proliferation in our myeloma cell lines.

To summarize, we found that the myeloma cell produced chemokine CCL27 exerts tumor progressive effects: In detail we found an induced migratory capacity, augmentation of the susceptibility to IL-6, and enhanced proliferation on the myeloma cells themselves. Concerning the effects on immune modulation, an impairment of dendritic cell function which results in decreased T cell activation potential and the impairment of dendritic cell migration could be observed.
All these findings led us to the conclusion that targeting CCL27 would be a potent tool for future antitumor therapies.

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DAMAGE OF HORMONAL FUNCTION AND BONE METABOLISM IN LONG-TERM SURVIVORS OF TESTICULAR CANCER

Dalibor Ondrus[1,2,3], Ladislav Dusek[2], Martina Ondrusova[2,3,4] and Beata Spanikova[5]

[1]1st Department of Oncology, Comenius University Medical School, St. Elisabeth Cancer Institute, Bratislava, Slovak Republic;
[2]Institute of Biostatistics and Analyses, Masaryk University, Brno, Czech Republic;
[3]National Cancer Registry, National Health Information Center; Bratislava, Slovak Republic;

[4]Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovak Republic;
[5]Out patient's Department of Osteology, St. Elisabeth Cancer Institute, Bratislava, Slovak Republic

Background: Improved survival of testicular cancer patients during recent years has led to rising interest on the disease consequences of the whole organism. Not only the tumor alone, but also its treatment may have an impact on a patient's hormonal status and bone metabolism. The aim of this study was to assign the hormonal profile and complete osteologic examination into algorithm of follow-up not only in patients with bilateral disease, but also in patients with unilateral testicular tumor. *Patients and Methods:* During the period of 11/2005-6/2008, we examined 828 patients diagnosed with testicular cancer after a mean follow-up of 87 months (range 9-260). Of these 776 patients were with unilateral (group A) and 52 with bilateral (group B) disease. Each patient was examined for hormonal profile (serum testosterone and LH level), marker of bone resorption – S-CTx (serum C-terminal cross-linking telopeptides of type I collagen) and serum calcium. Dual energy x-ray absorptiometry (DEXA) was performed on a Holoxic Explorer machine, focused on measurement of the hips and lumbar spine bone mineral density (BMD). Results of the osteological examination and hormonal profile examination were analyzed for associations with therapy following orchiectomy (orchiectomy alone, chemotherapy, radiotherapy, chemotherapy and radiotherapy) and with the time interval since the primary therapy. All key analyses were carried out separately for unilateral and bilateral tumors. Standard univariate statistical techniques were used to test the differences between groups of patients (ML chi-square test, one-way ANOVA). Unconditional logistic regression was used to relate potential risk factors to osteopenia and/or osteoporosis. All odds ratio estimates were adjusted for patient's age at the time of examination. *Results:* Group A: 776 patients with unilateral testicular cancer were followed-up for a mean 83 months (9-244) since therapy and were on average 39 years old (range 24-57) at the time of examination. Testosterone deficiency (<10.0 nmol/l) was observed in 34 patients (4.4%). Increased level of serum LH (>8.2 mU/ml) was observed in 122 patients (15.7%). Increased S-CTx was observed in 385 patients (49.6%). DEXA showed osteopenia and/or osteoporosis in 384 patients (49.5%). Serum calcium levels were in all cases normal. Group B: 52 patients with bilateral testicular cancer were followed-up for a mean 160 months (range 21-322) (average follow-up related to the 2nd tumor was 68 months, range 1-242) since the beginning of the therapy and were on average 41 years old (range 24-58). Testosterone deficiency was observed in 43 patients (82.7%). Increased level of serum LH was observed in 42 patients (80.8%). Increased S-CTx was observed in 33 patients (63.5%). DEXA showed osteopenia and/or osteoporosis in 38 patients (73.1%). Serum calcium levels were in all cases normal. Influence of age at examination and time to examination: Age at the time of examination was significantly increased in patients with osteoporosis (on average by 7 years) both in unilateral (p<0.001) and bilateral (p=0.022) tumors. The risk of osteoporosis increased with time since primary therapy (OR 1.08, p<0.05) and furthermore significant time cut-off points were found both for unilateral tumors (>8 years, OR 1.27) and bilateral tumors (>10 years, OR 3.90). In bilateral tumors, time >10 years since 2nd testicular cancer diagnosis was proven as an additional component increasing risk of osteoporosis (OR 2.85, 95% CI 1.07-7.61). Influence of primary therapy: In unilateral disease, applied CHT and/or RT increased significantly age-adjusted risk of osteopenia and/or osteoporosis (ORs ranged from 1.28 to 2.96). Application of radiotherapy further specifically increased age-adjusted risk of lumbal spine BMD impairment (OR 1.31; p<0.05). Radiotherapy was also significantly associated with increased TST and decreased LH levels at the time of examination in unilateral disease. In bilateral tumors, no significant association between therapy and risk of osteoporosis was found. This can be explained by highly significant risk potential of cancer bilaterality itself. Bilaterality reached highly significant odds ratio (p<0.001) for osteoporosis (3.32, 95% CI 1.48-7.42) and for osteoporosis + osteopenia (2.77; 95% CI 1.47-5.19). *Conclusion:* Examination of the hormonal profile and testosterone replacement therapy may be recommended as an important aspect of a patient's follow-up not only in bilateral disease (without consideration of the patient's sexual life), but also in patients with unilateral testicular cancer. The important part of a standard examination algorithm should also be an osteological examination to prevent osteopenia or even osteoporosis development. Particular attention should be paid to patients treated by radiotherapy.

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FUNCTIONAL STUDIES OF S100A6 USING PROTEOMICS

Lukas M. Orre[1], Erik Vernet[2], Johan Lengqvist[1], Torbjörn Gräslund[2], Rolf Lewensohn[1] and Janne Lehtiö[1]

[1]Karolinska Biomics Center (KBC), Department of Oncology and Pathology, Karolinska Institutet, Z5 plan 02, Karolinska University Hospital Solna, 171 76 Stockholm; [2]Department of Biotechnology, Royal Institute of Technology, KTH, Roslagstullsbacken 21, 106 91 Stockholm, Sweden

We previously used proteomic profiling to identify S100A6 as a protein up-regulated in response to DNA damage. Our studies also showed that S100A6 was expressed in a p53-dependent manner and that the post-translational modification pattern and subcellular localisation of S100A6 was altered

following irradiation. We and others have shown that S100A6 is overexpressed in a number of different tumor types. The connection between S100A6, cancer, p53 and DNA damaging treatment inspired us to investigate the cellular functions of S100A6 in depth.

As the functions of S100 proteins are believed to be through interactions with other proteins and regulation of these target proteins functions, we used proteomics to discover novel S100A6 interacting proteins. Immunoprecipitation using an S100A6 specific antibody was followed by proteomic analysis of the S100A6 precipitate using nano-LC MALDI-MS/MS. Using this approach we discovered Ubiquilin-1 as a novel S100A6 interacting protein.

To further investigate the function of S100A6 we generated stable S100A6 shRNA expressing lung cancer cell lines. These cell lines were then used in proteomics experiments to evaluate the effect of S100A6 silencing on the cellular proteome. Using this approach we were able to show connections of S100A6 with regulation of protein degradation, and in addition S100A6 silencing increased the cellular sensitivity to ionizing radiation.

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PRO AND CONS FOR SYSTEMATIC THERAPY IN RECURRENT OVARIAN CANCER?

Gülten Oskay-Özcelik, Werner Lichtenegger and Jalid Sehouli

Department of Obstetrics and Gynecology, Charite Medical University, Germany

The treatment of recurrent disease in ovarian cancer patients is an important aspect in the overall management. Ovarian cancer patients who do not respond to their initial chemotherapy or who relapse after achieving a response are generally incurable. Treatment goals after failure of first-line treatment for ovarian cancer are: (a) controlling or preventing disease-related symptoms, (b) maintaining quality of life with choosing an effective treatment with low toxicity potential and (c) prolonging progression-free survival. In contrast to the situation in previously untreated patients in whom prospective randomized phase III trials have established the current standard, in patients with recurrent disease there have been few randomized trials that have clearly demonstrated a survival advantage for a particular drug or regimen. In addition, a series of agents have been shown to have clinical activity in recurrent ovarian cancer, including topotecan, pegylated liposomal doxorubicin, gemcitabine, and oral etoposide. It has also been demonstrated that re-treatment with a platinum drug and taxane has been associated with significant clinical activity in patients with "sensitive" (treatment free interval >6 months) recurrent disease. The role of antihormonal therapy is unclear. However evidence-based treatment for recurrent ovarian cancer will require prospective randomized trials comparing efficacy, toxicity and quality of life.

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ROLE OF DNA CONTENT ANALYSIS AND IMMUNOHISTOCHEMISTRY IN THE EVALUATION OF THE RISK OF UNFAVOURABLE OUTCOME IN WILMS' TUMOURS

Maria-Chiara Osterheld, Liette Caron and Kathleen Meagher-Villemure

Institut Universitaire de pathologie, Bugnon 25, 1011 Lausanne-CHUV, Switzerland

Background: Wilms' tumour (WT) is the most common solid tumour affecting young children. Its histological diversity leads to difficulties in predicting the outcome. *Materials and Methods:* Image analysis cytometry and immunohistochemistry with a selected panel of antibodies were performed in 23 cases of WT considered intermediate risk tumours according to the revised International Society of Pediatric Oncology (SIOP) working classification of renal tumours of childhood. In this series, a tumour was considered aggressive according to its propensity for metastases or its recurrence. *Results:* Out of the 14 non-aggressive WT, 4 were found to be diploid and 10 were aneuploid, including 6 that were heterogeneous for DNA-ploidy. All the tumours presented a low proliferative index and were negative for p53 and p57kip2 immunostaining. Out of the 9 aggressive tumours, all were aneuploid and 4 were found to be heterogeneous for DNA-ploidy. They all presented a high degree of cell proliferation and 7 were positive for p53 immunostaining. Only two were positive for p57kip2 marker. The only fatal case revealed an aneuploid-homogeneous DNA-ploidy analysis, was p53- and $p57^{kip2}$- positive and presented a high cell proliferation index. *Conclusion:* A significant correlation between the presence of focal DNA-aneuploidy in Wilms' tumours and adverse prognosis was not established, but some immunohistochemical markers may be useful for the clinical evaluation of these tumours and to help in predicting the risk of unfavourable outcome.

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INTERSTITIAL RADIOSURGERY FOR BRAIN TUMOURS

Christoph B. Ostertag

Abt.Stereotaktische Neurochirurgie, Universtätsklinikum Freiburg, 79106 Freiburg i.Br., Germany

Interstitial radiosurgery is a local treatment modality and therefore aims at the treatment of localized tumours. Experimental interstitial radiosurgery produces well defined

small volumes of tissue necrosis which develop centrifugally from the implant and which are subsequently removed by macrophage activity. The perifocal toxicity is attributed to secondary effects, namely temporary changes in capillary permeability and regional cerebral blood flow. The damaged capillary surface area product increases with the square of the radius of the irradiated volume. The combination of temporary perifocal vasogenic edema and reduced blood flow in relation to volume limits the application of radiosurgery in the brain.

The clinical application requires the direct temporary placement of single or multiple radioactive sources in the form of seeds into the tumor volume. Tumour volume and target volume ideally are identical. Tissue inhomogeneities are of minor concern. The dose administered is confined with a steep dose gradient when compared with standard radiotherapy. Radioactive sources such as Iodine-125 (I-125) deliver the dose at a constant low-dose rate (1-100 cGy/h) compared with + 200 cGy/min by external beam radiotherapy or ± 5 Gy with focussed beam radiosurgery. As with other radiosurgical procedures, interstitial radiosurgery requires a precise knowledge of the relationship between the energy of the radioactive source, the radiation dose, the volume treated and the subsequent response of normal tissue surrounding the lesion. Only a small subgroup of patients with low-grade gliomas are appropriate candidates for interstitial radiosurgery, namely those with circumscribed tumors with only limited spread of tumor cells into the periphery. For this subgroup, which usually comprises not more than 25 % of all low-grade gliomas, interstitial radiosurgery competes with surgical resection. Interstitial radiosurgery is also be used to effectively treat gelastic epilepsy due to hypothalamic hamartomas with less invasiveness and side-effects compared with open surgery.

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A REVIEW ON FIRST-GENERATION HEPOXILIN ANALOGS (PBTs) AS CANCER THERAPEUTICS

Cecil R. Pace-Asciak

Programme in Physiology and Experimental Medicine, Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8 (primary address) and Department of Pharmacology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8

In this review, the utility of stable first-generation analogs (PBT) of the hepoxilins in controlling the growth of neoplastic cells is discussed from *in vivo* xenograft animal studies (nude mice) and from *in vitro* studies. The PBTs cause apoptosis of solid tumors in leukemic (human CML) and human breast cancer animal models *in vivo* with a threshold effect at 1.2 mg/kg in the CML model. The compounds are well tolerated even at doses 10×above threshold, are stable and have long-term efficacy (up to 50 days) after an 8-day treatment. This delay in tumour growth can be doubled if treatment is carried out during two interrupted 8-days periods. The PBTs are active *in vitro* on several human neoplastic cell lines tested derived from chronic myelogenous leukemia (K562), breast cancer (MDA MB231, MCF-7 and MT-3), prostate cancer (DU-145), primary keratinocytes (HPK) and cervical cancer (HeLa). The PBTs have no effect on normal cells (3T3-L1 and smooth muscle). The PBTs are effective in causing apoptosis of K562 cells that were resistant to Gleevec. These studies point to an effective PBT base structure which may allow modification to afford second-generation compounds with improved pharmaceutical properties as cancer therapeutics with minimal or no side-effects.

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FORTY YEARS WITH SERUM CANCER MARKERS. A RETROSPECTIVE AND PROSPECTIVE ANALYSIS

Michel Pagé

Faculty of Medicine, Université Laval, Québec, Canada

The first serum cancer markers, such as alphafoetoprotein and carcinoembryonic antigen, described more than forty years ago are also present at various developmental stages of the foetus or the embryo. Following the enthusiasm of the first reports on the sensitivity and specificity of these markers, elevated concentrations of these proteins were soon reported in certain benign diseases. Many new markers, such as CA 125, CA 15.3, CA 19.9, were developed following the advent of monoclonal antibodies and these are still in use today. Prostate-specific antigen (PSA) was rapidly accepted as a serum marker for this cancer. After a few years of experience with these markers, it became evident that these assays were neither specific nor sensitive enough to be used for cancer screening but they could be used efficiently for the follow-up of treated patients. Recently developed markers are now intended for individual therapy. More recently, serum proteins and peptides have been analyzed by protein array or MALDI-TOF-MS to obtain a profile and predict the outcome of targeted therapies. With the advent of these new technologies, the clinician may not only confirm a diagnosis but may soon be able to plan the best treatment and predict the most probable outcome.

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BIOTINYLATED CISPLATIN-LOADED PAMAM DENDRIMERS FOR CHEMOTHERAPY OF OVARIAN CANCER

Venkata K. Yellepeddi, Ajay Kumar and Srinath Palakurthi

Irma Lerma Rangel College of Pharmacy, Texas A&M Health Science, Center, Kingsville, Texas-78363, USA

Objective: Biotin, also known as vitamin H, is a growth promoter of cells and biotin levels were found significantly higher in cancer cells as compared to normal tissue. Rapidly proliferating cells need higher amounts of biotin. We hypothesized that conjugation of biotin to polyamidoamine (PAMAM) dendrimers and their use as carriers for cisplatin therapy would provide targeted therapeutic effect and reduce the side effects of cisplatin. Cisplatin-loaded biotinylated dendrimers of different generations were used and their encapsulation efficiency, *in vitro* release profile and *in vitro* cellular uptake were studied. *Materials and Methods:* Dendrimers were biotinylated using Sulfo-NHS-LC-Biotin. Cisplatin was loaded into the biotin dendrimer conjugates by aqueous reaction for 4 hrs and unencapsulated cisplatin was separated by column chromatography. Encapsulation efficiency and *in vitro* release of cisplatin loaded biotin dendrimers was performed using HPLC. Cytotoxicity of cisplatin-dendrimer conjugates in OVCAR-3, SKOV, A2780 (wild-type) and CP 70 (cisplatin resistant) cell lines was assessed using MTT (methylthiazole tetrazolium) assay and the results were compared with simple cisplatin. Cellular uptake of the dendrimers was studied in A2780 and CP70 cell lines using HPLC. Statistical analysis of the data was performed using ANOVA and $p<0.05$ was considered significant. *Results and Discussion:* The biotin-dendrimer (G4 PAMAM-NH_2 and G4-PAMAM-COOH) conjugates were synthesized and characterized using ^1H NMR and MALDI spectral analysis. Encapsulation efficiency biotin-G4-NH_2 and biotin-G4-COOH dendrimers was 1.97% and 10.85% respectively. Cytotoxicity of biotinylated dendrimers (IC_{50}-34.44 μM) was 3 fold higher than free cisplatin (IC_{50}-106.21 μM), validating our hypothesis that these conjugated dendrimers can be used for targeting ovarian cancer with minimum amount of cisplatin required to elicit desired cytotoxic activity. *In vitro* cellular uptake experiments in CP70 showed significantly higher cisplatin levels as compared to cisplatin ($p<0.01$).

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A NOVEL NONSENSE MUTATION IN KRAS GENE

Raffaele Palmirotta[1], Annalisa Savonarola[1], Vincenzo Formica[2], Giorgia Ludovici[1], Roberta D'Alessandro[1], Barbara Leone[1], Marco Ciancia[1], Raffaella Liberatore[1], Stella D'Angelo[1], Mario Roselli[2] and Fiorella Guadagni[1]

[1]Laboratory of Molecular Diagnostics, Department of Laboratory Medicine and Advanced Biotechnologies, IRCCS San Raffaele Pisana, Via Della Pisana 235, 00163 Rome; [2]Medical Oncology, "Tor Vergata" Clinical Center, University of Rome "Tor Vergata", Rome, Italy

Cancer development and progression is a multi-step process based on the accumulation and clonal selection of somatic mutations in key cancer-related genes, corresponding to activation of oncogenes and inactivation of tumor suppressor genes. One of the most well studied cellular genes involved in the pathogenesis of human cancer is the K-ras proto-oncogene, which encodes a 21-kDa GTP-binding protein that controls the mechanisms of cell growth and differentiation. Point mutations in the K-ras gene lead to uncontrolled stimulation of ras-related functions by the altering p21 ras protein-related pathway. Mutations in the K-ras oncogene are frequently found in human cancers, such as colorectal cancer, pancreatic cancer, lung adenocarcinoma, gall bladder cancer, bile duct cancer and thyroid cancer. Activating mutations occur in hot-spots mainly of codons 12 and 13. These mutations may be predictive of drug response and can also indicate prognosis. In particular, recent publications have shown that the successful treatment of metastatic colorectal cancer (mCRC), using monoclonal antibody therapies such as Panitumumab is directly linked to the oncogenic activation of the K-ras signaling pathway. In our laboratory, molecular analysis of K-ras is routinely carried out on genomic DNA extracted by paraffin-embedded tumor samples after microdissection. Exons 1 and 2 of K-ras are individually amplified by PCR using specific primers for the K-ras gene and then sequencing analysis is performed. Here, we report a case of a patient with mCRC, who referred to our laboratory in order to evaluate K-ras somatic cancer mutations. Molecular analysis performed on paraffin-embedded samples showed a mutation in the first exon of the K-ras gene (CAG>TAG), that determines a premature stop signal at codon 22 (Gln22Stop). Such inactivation of K-ras gene is a mechanism not reported before, suggesting a possible involvement of other genes or epigenetic factors. Indeed, all mutations reported in literature, to date, are hot-spots missense point mutations at codons 12, 13, 59, 61, 117, while recently a novel 15-bp insertion, resulted in tandem duplication of codons 62-66, was reported. There are no other reports that describe mutations of K-ras gene resulting in a premature stop signal. These data help to clarify the importance to evaluate K-ras mutation, in order to identify not only the molecular mechanisms of cancer progression but also subjects who are likely to benefit from targeted therapies and to avoid costly and potentially toxic treatments in non-responder patients.

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IS BREAST CANCER AN INTEGRAL BHD-RELATED TUMOR?

R. Palmirotta[1], A. Savonarola[1], G. Ludovici[1], P. Donati[2], A. Spila[1], B. Leone[1], M. Ciancia[1], L. Polce[1], M.G. Pera[1], P. Ferroni[1] and F. Guadagni[1]

[1]Department Of Laboratory Medicine and Advanced Biotechnologies, IRCCS San Raffaele Pisana, Rome;

[2]Unit Of Skin Histopathology, IRCCS San Gallicano Dermatologic Institute, Rome, Italy

The Birt-Hogg-Dubé syndrome (BHD) (OMIM 135150) is a rare autosomal dominant genodermatosis predisposing patients to develop multiple, small papules on the face, neck, and upper thorax after the age of 25 years. These lesions, called "fibrofolliculomas" (FFs), are histologically classified as benign hamartomas of the hair follicle. The syndrome is caused by germline mutations in the folliculin (FLCN) gene (also known as BHD–OMIM607273) which encodes a tumor-suppressor protein. Numerous mutations have been described in the FLCN gene, the most frequent occurring within a C8 tract of exon 11, resulting in a truncated folliculin; this "hot spot" mutation has been found in the germline of 44% BHD patients. This hypermutability is probably due to a "slippage" in the DNA polymerase during DNA replication, resulting in gains or losses of repeat units, as happens for other genes causing cancer predisposition. However, recent reports suggest that all translated exons might be mutated.

The main phenotypic manifestations related to this disease, as reported in the literature, are lung cysts leading to pneumothorax, and an increased risk for renal neoplasia. Also discussed is the genotype/phenotype correlation between FLCN mutations and risk of colon cancer. While some reports describe this correlation, a recent risk-assessment study of BHD-affected patients concluded that a diagnosis of BHD conferred a 7-fold increased risk of developing renal neoplasia and a 50-fold increased risk of spontaneous pneumothorax but no increased risk of colon polyps or colon cancer.

Other reports describe additional phenotypic manifestations in BHD-affected individuals, including lipomas, angiolipomas, parathyroid adenomas and parotid oncocytomas.

Among the BHD-affected families referring to our Institutions, we selected two families; genetic counseling was performed in order to obtain family trees and molecular analysis of the FLCN gene was performed. The proband of the first family had a history of breast cancer at the age of 44 and of colon cancer at the age of 56; molecular analysis showed the occurrence of a frameshift mutation, not previously reported, located in exon 9 (1345delAAAG) of the FLCN gene. 1345delAAAG was associated with a wide variety of tumors, including stomach, colon and parotid cancer found in other family members. The proband of the second family had a history of breast cancer at the age of 42; molecular analysis showed the occurrence of a missense mutation in exon 12 (G1788A). Studies are currently ongoing to assess the occurrence of a possible "second hit" somatic mutation in breast cancer tissues. To date, there are no reports describing a possible association between germline FLCN mutation and risk of breast cancer. The genes so far associated with hereditary forms of breast cancer are BRCA1 and BRCA2, while a small fraction of risk can currently be attributed to germline mutations in other genes, such as p53, mismatch repair genes, ATM, PTEN and LKB1. Our data, in association with the early age of onset of breast cancer in patients with mutations in the FLCN gene, suggest for the first time the possible association between germline FLCN mutation and risk of breast cancer.

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ALL TRANS-RETINOIC ACID REVERSES THE PROTUMORAL EFFECT OF HEPATOCYTE GROWTH FACTOR ON THE HIGHLY METASTATIC S4MH RABDOMYOSARCOMA CELL LINE

Teodoro Palomares, Roberto Sanisidro, Ignacio García-Alonso and Ana Alonso-Varona

Departments of Surgery and Radiology and Physical Medicine, and Cell Biology and Histology, School of Medicine and Dentistry, University of the Basque Country, Leioa, Vizcaya, Spain

Purpose: We previously demonstrated the tumour-enhancing effect derived from hepatic resection of liver metastases, which could be exerted through growth factors (GF) related with liver regeneration, such as hepatocyte growth factor (HGF). The aim of this work was to study the role of HGF on the growth of the highly metastatic S4MH rabdomyosarcoma (RMS) cell line, and to analyze the possible preventive effect of all-*trans*-retinoic acid (ATRA) on the HGF pro-tumour effect. *Materials and Methods:* The poorly differentiated and highly metastatic S4MH RMS cell line was used. Cells were cultured in 24-well microplates at a density of 10^4 cells/well in the presence or absence of different concentrations (5-40 ng/ml) of HGF, in order to determine the optimal concentration of HGF on cell proliferation. Afterwards, ATRA (10^{-6} M) or the control solvent was also administered every 48 h to cells cultured in presence of HGF, and the chemopreventive effects of ATRA on the pro-tumour effect of this GF was analyzed. Proliferation was measured by using a haemocytometer. *Results:* HGF significantly enhanced cell proliferation in a concentration-dependent manner up to a dose of 10 ng/ml. Higher concentrations were toxic. In the presence of 10 ng/ml of HGF, the number of cells at 24 and 72 h were 1.3 and 1.2 times higher than in control cultures. The increase in proliferation rate induced by HGF was mainly apparent in the first 24 h of culture (1.3 times higher than that of the controls); afterwards, this rate was similar in HGF and control cultures (proliferation rate close to 2.5 at 48 and 72 h). ATRA significantly reduced (1.3- and 1.2-fold at 24 and 72 h, respectively) the growth of S4MH cells. Moreover, ATRA also significantly reduced the pro-tumour effect of HGF; thus, with respect to cells cultured with HGF alone, ATRA treatment

reduced cell proliferation 1.8- and 1.7-fold at 24 and 72 h, respectively ($p<0.05$). *Conclusion:* These results suggest that ATRA could be a useful chemopreventive agent to prevent the pro-tumour effects of HGF on RMS.

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L-2-OXOTHIAZOLIDINE-4-CARBOXYLATE REVERSES THE TUMOUR GROWTH-PROMOTING EFFECT OF THE GROWTH FACTORS HGF, VEGF AND EGF ON HUMAN COLON CANCER WIDR CELLS

Teodoro Palomares, Marta Caramés and Ana Alonso-Varona.

Departments of Surgery and Radiology and Physical Medicine, and Cell Biology and Histology, School of Medicine and Dentistry, University of the Basque Country, Leioa, Vizcaya, Spain

Purpose: Gluthathione (GSH), the most prevalent intracellular non-protein thiol, is involved in the growth factor-induced proliferative activity. The present study investigated the effect of manipulation of GSH using the cysteine prodrug L-2-oxothiazolidine-4-carboxylate (OTZ) on the response of colon cancer cells to growth factors (GF). *Materials and Methods:* A highly metastatic human colon cancer WiDr cell line was selected. Cell were seeded in 24-well microplates at a density of 10^4 cells/well and allowed to grow for 24 h. The cells were treated with OTZ for 4 h. Afterwards, the cells were exposed to hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF). Proliferation was measured by using a haemocytometer. In the GSH assay, the cells were stained with 100 μM monochlorobimane and the GSH content was determined using the CytoFluor-2350 system. *Results:* The three GFs significantly enhanced cell proliferation. In the presence of 7.5 ng/ml of HGF, 10 ng/ml of VEGF and 25 ng/ml of EGF, the number of cells at 48 h was 1.7, 1.2 and 1.2 times higher, respectively, than in control cultures. Whereas exposure of cells to VEGF and EGF resulted in a 50% reduction in GSH levels after 1 h of incubation and increased them at 2 h (50% increase compared with control cells), the addition of HGF for 2 h produced a significant GSH depletion (30%) and a 45% increase after 4 h compared with non-exposed cells. However, treatment with 5 mM OTZ produced a 42.6% reduction in the cellular GSH content after 4 h of incubation and a 1.3-fold reduction in the growth rate. Moreover, exposure to OTZ abrogated the pro-tumour effects of the GFs. Thus, OTZ treatment reduced by 1.6-fold the growth rate of cells in the presence of HGF and VEGF and by 1.4-fold in the case of EGF with respect to untreated cells at 72 h. *Conclusion:* OTZ is capable of reversing the growth-promoting effects of GFs. This fact could be important in the design of therapeutic strategies involving intracellular GSH level modification as a mechanism for tumour cell sensitization to cytotoxic drugs.

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CONSTITUTIONAL STRUCTURAL CHROMOSOMAL ABNORMALITIES AND HEMATOLOGICAL MALIGNANCIES

Anna D. Panani

Critical Care Department, Medical School of Athens University, Cytogenetics Unit, Evangelismos Hospital, Athens, Greece

Over the past four decades, it has become clear that acquired recurrent chromosomal changes are associated with specific malignant diseases. Among acquired structural chromosomal abnormalities, reciprocal translocations have been identified in a variety of malignant diseases, mainly including leukemias, lymphomas and sarcomas and they have been implicated in the etiology of the neoplastic process with involvement of specific genes. On the other hand, it is now well recognized that certain constitutional chromosomal aberrations confer a tumor predisposition.

The observation that constitutional structural chromosomal aberrations are associated with a predisposition to cancer has led to a two-hits hypothesis for cancer development. The first hit is a constitutional abnormality involving a specific gene and the second hit is an acquired inactivation of the other allele by mutation or other genetic change. Investigations of families with hereditary cancer and constitutional chromosomal abnormalities have been key observations leading to the molecular identification of specific genes implicated in tumorigenesis, such as the loci involved in retinoblastoma patients with 13q deletion and the loci in Wilms tumor patients with 11p deletion. Large studies have been reported on the incidence of constitutional chromosomal aberrations in patients with hematological malignancies, but they could not confirm an increased risk for hematological malignancy among carriers of structural chromosomal changes. However, it is of particular interest that constitutional structural chromosome aberrations with breakpoints similar to leukemia-associated specific breakpoints have been reported in patients with hematological malignancies. This might indicate that the constitutional anomaly itself probably plays a role in the neoplastic process.

There is substantial discussion in the literature about mechanisms involved in constitutional structural chromosomal abnormalities. The documentation of more patients with hematological malignancies and constitutional structural chromosomal changes could be of major importance. Most importantly, the molecular cloning of the

chromosomal breakpoints involved in constitutional rearrangements in patients with hematological disorders could give useful information on the genetic events underlying constitutional anomalies contributing to isolation of genes important in the development of the neoplastic process.

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THE GROWTH INHIBITORY EFFECTS OF CADMIUM AND COPPER ON MDA-MB468 HUMAN BREAST CANCER CELLS

Mojtaba Panjehpour, Masih-Allah Taher and Mortaza Bayesteh

School of Pharmacy and Isfahan Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Cadmium chloride is an important occupational and environmental pollutant but under certain conditions can also be anticarcinogenic. Copper is an essential trace element. Moreover it was shown that apoptotic potential of copper is associated with its ability to generate reactive oxygen species. The aim of this study was to determine the ability of cadmium chloride and copper chloride to cause cell death in a human breast cancer cell line (MDA-MB-468). *Methods:* MDA-MB-468 cells were grown in RPMI-1640 medium supplemented with 10% FCS, Penicillin/ streptomycin (100 U/ml, 100 μg/ml) at 37˚C in 5% CO_2/95% air. The cells were plated in 96-well plates. After 24 hours, different concentrations of cadmium chloride and copper chloride were added to plates which were then incubated for 48 and 72 hours. MTT cell viability test was used to study the cytotoxic effects of cadmium and copper. *Results:* Exposure of monolayers to different metal concentrations (1-1000 μM) for different times showed a significant decrease (p<0.05) of viable cells when compared with that of controls in a dose-dependent manner; a significant cytotoxicity was observed at 200 μM for cadmium chloride at 48 hours and 1 μM at 72 hours. For copper chloride, significant cytotoxicity was best observed at 1000 μM at 48 hours and 1 μM during 72 hours. The maximum synergic cytotoxic effect was observed at 0.5 μM cadmium chloride and 0.5 μM copper chloride during 72 hours exposure. *Conclusion:* In this study it has been shown that there is differential sensitivity of the cell line to the antitumor activity of cadmium chloride and copper chloride. The results of the present study also indicated that the cytotoxic effect of copper chloride is somewhat less than that of cadmium chloride. This may be due to vital physicological role of copper, none of which are known for cadmium as yet. In other words, copper is used for natural consumption by the cells also. Altogether, these findings may suggest a new view on the mode of action and possible application of trace elements in cancer treatment.

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THE ROLE OF THE GROWTH FACTOR PLEIOTROPHIN AND ITS RECEPTORS IN TUMOR GROWTH AND ANGIOGENESIS

Evangelia Papadimitriou, Constantinos Mikelis, Evgenia Lampropoulou, Marina Koutsioumpa, Katerina Theochari, Sotiria Tsirmoula, Christina Theodoropoulou, Margarita Lamprou, Evanthia Sfaelou, Dionyssios Vourtsis and Panagiotis Mpountouris

Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Patras GR26504, Greece

Pleiotrophin (PTN), also known as heparin affin regulatory peptide or heparin binding growth associated molecule, is an 18 kDa growth factor that has high affinity for heparin, and together with midkine forms a family of structurally related heparin binding growth factors. The two proteins share 45% homology in their amino acid sequence and many, but not all, biological activities. The first described biological activity of PTN is stimulation of neurite outgrowth and a role in the growth and maturation of brain. PTN also induces proliferation of several types of cells, is involved in a variety of processes in bone formation, seems to play a critical role in chondrogenesis and participates in normal spermatogenesis and fertility. Screening of various human tumour cell lines and tumour speciments of different origin revealed that PTN is expressed in many types of cancer, such as gliomas, melanomas, meningiomas, neuroblastomas, choriocarcinomas, leukemias and cancer of pancreas, prostate, stomach, colon, breast, ovaries and lungs. Concerning the biological activity of PTN in cancer, there is ample evidence that it is a tumor-promoting factor, enhancing tumor cell proliferation, migration, anchorage-independent growth and angiogenesis *in vivo* or *in vitro*. PTN receptors are also up-regulated in a plethora of tumors and are being tested as targets for anticancer therapy. We have recently identified $\alpha_v\beta_3$ integrin as an important mediator of PTN-induced endothelial and tumor cell migration and the molecular mechanisms involved in this pathway, as well as its involvement in tumor growth and angiogenesis are being investigated. We are also working on the regulation of PTN expression, as well as structure–function relationships, knowledge that could lead to identification of new target(s) and the possible development of new therapeutic tools.
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NLCQ-1 (NSC 709257): A WEAK DNA-INTERCALATING BIOREDUCTIVE DRUG. NEW PROSPECTS

M.V. Papadopoulou, W.D. Bloomer, B. Fitzpatrick, R.L. Cowen, I.J. Stratford and K.J. Williams

Evanston Northwestern Healthcare, Evanston, IL, USA and University of Manchester, UK

Numerous studies have confirmed the value of the bioreductive compound NLCQ-1 as a tumor hypoxia-targeting agent and as an enhancer of radiotherapy, radioimmunotherapy and chemotherapy. In addition, studies using rat liver microsomes have suggested a role for cytochrome p450 reductase (P450R) in NLCQ-1 bioactivation. As human tumor levels of P450R are heterogeneous and not substantially elevated *versus* those of normal tissue, exploitation of P450R in a therapeutic context requires a gene therapy-based approach unless other enzymes overexpressed in tumors, such as inducible nitric oxide synthase (iNOS) can be exploited in the activation of NLCQ-1. Therefore, in our recent studies we investigated the effect of NLCQ-1 in tumors overexpressing P450 reductase, DT-diaphorase (DTD) and iNOS. In addition, the ability of NLCQ-1 to prevent tumor-micrometastasis, alone or in combination with radiotherapy was evaluated. Finally, we have investigated the effect of NLCQ-1 against resistant, latent TB-bacilli, since TB latency is related to anaerobiosis.

MDA231 (breast) and HT1080 (fibrosarcoma) cells were stably transfected to overexpress P450R, DTD or iNOS and exposed to NLCQ-1 in air or hypoxia (3 h) 48 h post-transfection. NLCQ-1 cytotoxicity was determined 3-days later by MTT assay. Metastatic studies were conducted in mice bearing subcutaneous KHT tumours (250 mm^3). Tumors were irradiated with a single dose of 25 Gy whereas NLCQ-1 was administered 72 h post-radiotherapy (10 mg/kg/day × 4). Metastatic dissemination to the lungs was analysed 21 days post radiotherapy. Antitubercular activity was evaluated in H37Rv bacteria by using the luminescence-based low oxygen recovery assay (LORA) and toxicity was assessed in Vero cells by using the MTT assay.

Improved hypoxic selectivity was observed for NLCQ-1 in P450R and iNOS transfected cells, suggesting a therapeutic advantage of combining NLCQ-1 with gene therapy. NLCQ-1 proved very efficacious in the metastatic studies, with 7/9 animals showing no visible signs of lung metastasis and in addition it improved tumor local control. Finally, NLCQ-1 was selectively active against latent TB mycobacteria, normally localized in the lungs. These data suggest that NLCQ-1 not only can prevent metastatic lesions in the lungs as an adjuvant to radiotherapy but also can be beneficial in combination with gene therapy and in the treatment of latent tuberculosis.

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LIPID CORE MICELLES FOR *IN VIVO* WHOLE BODY TUMOR TARGETING AND IMAGING

A. Papagiannaros, D. Chovatia, J. Upponi, P. Shah, A. Kale, D. Mongayt, W. Hartner, T. Levchenko and V. Torchilin

Department of Pharmaceutical Sciences and Center for Pharmaceutical Biotechnology and Nanomedicine, Northeastern University, 312 Mugar Life Sciences Building, Boston, MA 02115, USA

Introduction: Whole body *in vivo* imaging of nanoparticles, macromolecules, quantum dots and living cells can be carried out in the near infrared region of the spectrum and offers results with high sensitivity and precision. This method's advantages over more conventional techniques, such as the use of radioisotopes, include easier handling, improved versatility and cost effectiveness. This IR whole body imaging is noninvasive, and the images are captured in real time. The quantification of the bio-distribution of the micelles was only semi-quantitative at best, due to the intense scattering and absorption of the fluorescence. The aims of the study are to use near infrared whole body imaging to visualize and quantify the bio-distribution of nanoparticles, and to develop nanosized contrast agents for the detection of cancer and disease sites with high sensitivity and precision. *Materials and Methods:* Micelles encapsulating Near Infra Red emitting Quantum dots (Qdot800) or Alexa 750-PE were produced and their size distribution was estimated by dynamic light scattering (Beckman Coulter N4). Actively targeted quantum dot micelles were also produced by direct conjugation of the anti-nucleosome specific antibody 2C5 to PEG-PE. Tumor bearing animals (1.5×10^6 4T1 cells, *s.c.*, right flank) were anesthetized and micelles or quantum dots were injected via the tail vein. Mice were visualized in a Kodak IN VIVO IMAGE STATION FX (excitation filter 720 nm, emission 790 nm). The fluorescence from the lipid nanoparticles was compared with commercially available formulations. Images were analyzed using the Kodak Molecular Imaging Software or NIH ImageJ. *Results and Discussion:* Tumor fluorescence in mice injected with the quantum dot micelles was higher than the commercially available pegylated quantum dots, so the images were sharply contrasted. The signal form the lipid coated quantum dots maximized within one hour from the injection while the fluorescence from commercially available pegylated quantum dots reached similar levels after four hours. Overall the signal of the lipid QD micelles was higher while to dose is half. The variability of the bio-distribution was smaller in the case of the lipid coated nanoparticles and the cost was also smaller. The lipid contrast agent seems to accumulate to the tumor, liver, spleen and kidneys. The results of the quantification for the micelles were similar to those previously reported using radioisotopes. The actively targeted

nanoparticles accumulation to the tumor was double that of the non targeted quantum dot micelles, producing even more sharp images at one hour after the injected. Alexa encapsulated micelles contrast was high enough to localize readily the tumor within one hour, but afterwards the contrast of the images decreased due to a high abdominal signal, due to the secretion of the lipids. *Conclusion:* We have developed an optical molecular imaging system in the near infrared spectrum for the *in vivo* visualization of nanoparticles bio-distribution and for the imaging of tumors. The active targeting of the quantum dot immuno-micelles in metastatic tumor models is under investigation.

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PALLIATIVE CARE IN GERMANY

Jens Papke

Praxis for Internal Medicine and Oncology, Rosa-Luxemburg-Strasse 6, D – 01844 Neustadt/Sachsen, Germany

The history of palliative care (PC) in Germany is not very long. The hospice movement from Great Britain has influenced German medicine since the 1970s. In this "decade of ignorance" nothing more happened in Germany. In the 1980s, the "decade of the pioneers", the first department for PC of a hospital started in Koeln in 1983 and the first hospice opened in Aachen in 1986. In the following "decade of establishment", as in other European countries, many hospices and PC services started working and developed. For example, the outpatient PC project "Home Care Berlin", a specialised medical PC service working since 1994, cares for more than a thousand patients every year. Data and experiences of this service are presented and compared with data from international PC services.

In 1994, the German Society of Palliative Medicine was founded, the first professorship in PC was founded in 1999. In this time, the civic hospice movement started in Germany and the number of hospices and PC units in hospitals grew rapidly.

In the last year, the government passed a bill for reformation of the health care system. Part of this bill was the acceptance of PC as a part of obligatory health insurance. Now, very different models are developed to provide and improve PC in the country. One model, the Home Care Sachsen Association, is presented with first data. *Conclusion:* After a quarter of a century PC in Germany is still developing. Nationwide provision has still not been achieved. Models for PC differ regionally and structurally. Current strategies aimed at standardising the concepts have to be reconsidered.

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A MOUSE MODEL OF HUMAN HEAD AND NECK SQUAMOUS CELL CARCINOMA THROUGH THE SOMATIC ACTIVATION OF AKT AND TP53 DEFICIENCY

Marta Moral, Carmen Segrelles, Corina Lorz, Ramón García-Escudero, Mirentxu Santos, Mª Fernanda Lara, Cristina Saiz, Ana Belén Martínez-Cruz, Clotilde Costa, Águeda Buitrago and Jesús M. Paramio

Molecular Oncology Unit. Division of Biomedicine, CIEMAT. Ave. Complutense 22, E28040 Madrid, Spain

Akt/PKB is a key element in the PI3K/PTEN/Akt pathway that is overactivated in many tumor types including head and neck squamous cell carcinoma (HNSCC). Here we show that transgenic mice expressing a constitutively active form of Akt in the basal layer of stratified epithelia, using the control of keratin K5 promoter sequences (K5MyrAkt), develop pretumoral lesions and carcinomas *in situ* in the oral cavity which resemble human oral dysplasias and *in situ* carcinomas. However they do not progress into advanced aggressive carcinomas due to the induction of p53-dependent senescence. Accordingly, a transgenic mouse line in which active Akt is combined with the somatic deletion of the *TP53* gene in stratified epithelia ($p53^{F/F}$K14Cre;K5myrAkt mice) develops malignant aggressive oral tumors. These tumors display multiple molecular and histopathological characteristics that phenocopy human oral SCC and can be followed through *in vivo* imaging by PET. Overall, these models should help in understanding the pathogenesis of human HNSCC and will likely prove useful tools for preclinical testing of therapies targeting the Akt and p53 signaling pathways.

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TP53 LOSS IN EPIDERMIS GENERATES AGGRESSIVE METASTATIC TUMORS AND PROVIDES A GENOMIC TOOL FOR PREDICTION OF CLINICAL OUTCOME OF HUMAN CANCER

Ramón García-Escudero, Ana-Belén Martínez-Cruz, Mirentxu Santos, Carmen Segrelles, Marta Moral, Corina Lorz, Clotilde Costa, Águeda Buitrago-Pérez, Cristina Saiz-Ladera and Jesús M. Paramio

Molecular Oncology Unit. Division of Biomedicine, CIEMAT, Ave. Complutense, 22, E-28040 Madrid, Spain

Squamous cell carcinomas represent the most aggressive type of non-melanoma skin cancer. Here we report that using the Cre-loxP system, loss of p53, but not pRb, in epidermis leads to the development of spontaneous tumors, whose occurrence is severely accelerated in doubly deficient mice. The tumors are aggressive, undifferentiated and display a hair follicle

origin. Moreover they also display a high metastatic potential to the lungs. Using expression profiling and statistical tools for transcript set enrichment analysis, we performed a cross-species comparison of epidermal p53-deficient models and human cancer samples. The results demonstrated that the mouse p53 tumors recapitulate molecular features of p53-mutated human cancer samples, undifferentiated and aggressive human tumors from different anatomical locations, and human embryonic stem cells. In addition, we demonstrated that mouse p53-deficient primary tumors contain molecular determinants for metastatic progression, which has allowed us to develop a 40-gene predictor for human breast cancer clinical outcome. Our gene profiling analyses validate mouse p53 tumor models as tools for preclinical tests of targeted therapies against human aggressive tumors. Moreover, we demonstrated that animal models with aggressive cancer can be used to develop genomic predictors for clinical outcome of human cancer.

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RHEUMATOID ARTHRITIS: CORRELATION BETWEEN RHEUMATOID FACTOR LEVELS AND CA-125 TUMOUR MARKER ELEVATION

N. Tsavaris, C. Mavragani, A. Ntokou, J. Syrios, Th. Paraskevopoulos and D. Pikazis

Department of Pathophysiology, Oncology Unit, Athens University School of Medicine, Laikon General Hospital, Athens, Greece

Objectives: We aimed at examining whether patients with RF-positive rheumatoid arthritis and absence of clinical or laboratory evidence of a neoplastic disorder could have "falsely" elevated levels of some commonly applied serum tumor markers. *Methods:* Patients fulfilling the American Rheumatology Association (ARA) diagnostic criteria for rheumatoid arthritis entered the present study. Data were collected for patients with rheumatoid arthritis who had increased indices of ovarian cancer (CA-125>33 U/ml) without the presence of cancer. Moreover, data were also collected for the following variables: RF-test, CRP, and serum tumor marker levels; CEA, and CA-19.9 measured by ELISA. Relationships between ordinal variables were studied with the use of Spearman non-parametric tests. *Results:* Fifty-three consecutive patients fulfilling the diagnostic criteria of rheumatoid arthritis were entered; 45 women and 8 men, with a median age 51 years (range, 23-58). The associations between Ra-test and CRP with CEA, CA-125 and CA-19.9 were studied and non-significant relationships were detected between Ra-test, CRP and any of the tumor markers. Two patients (3.8%) had high values of CEA and 7 patients (13.2%) of CA-125. There was one patient (1.9%) found with high value of CA-19.9 index. No significant relationship was detected between Ra-test and CEA, CA-125 while a near significant correlation was found between CRP and CA-125 (p=0.08). No significant relationship was detected between Ra-test and CEA, CA-125, CA-19.9. None of the patients included in the present study developed cancer after a minimum period of 3-year follow-up. *Conclusion:* The present report raises the issue of serum tumor marker, in particular CA-125, false elevation in the presence of circulating RFs. However, none of the patients appear to develop cancer after adequate follow-up. It is expected that future studies should attempt to develop methods eliminating RF binding.

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STUDY OF CYTOKINES BYSTANDER SIGNALLING IN HUMAN GLIOBLASTOMA CELLS AFTER EXPOSURE TO GAMMA RADIATION

F. Pasi[1,3], A. Bertolotti[2,3], A. Facoetti[2,3], L. Mariotti[2,3], A. Ottolenghi[2,3] and R. Nano[1,3]

[1]Department of Animal Biology, University of Pavia;
[2]Department of Nuclear and Theoretical Physics, University of Pavia;
[3]INFN Pavia, Italy

Radiation-induced bystander effect is defined as the induction of biological effects in cells that are not directly traversed by radiation but are receiving signals from the irradiated cells that are in close proximity to them. Although the bystander effects have been well described over the past decade, the precise mechanisms of the process remain unclear. Soluble extracellular factors which are released from irradiated cells are involved in bystander responses and in particular cytokines are considered to be good candidates for signalling between irradiated and non-irradiated cells. The aim of this study was to investigate the modulation of intercellular communication, mainly mediated by alteration of cytokine release into the culture medium and of its receptor expression in human glioblastoma cells (T98G) after exposure to gamma radiation. For this purpose, we used the ELISA technique to characterize the time-and dose-dependence of concentration of IL6, IL8 and TGFβ in the culture medium of sham irradiated and irradiated cells. In parallel, the expression of the corresponding cell membrane receptors was evaluated by immunocyto-chemistry both in irradiated glioma cells and in cells cultured with medium collected and filtered from irradiated cultures. The results suggest that gamma radiation affects the release kinetic of cytokines, each with particular features, from irradiated cells and the receptor profiles in irradiated and bystander cells.

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RENAL CANCER: PATHOLOGICAL DIAGNOSIS AND MOLECULAR CHARACTERIZATION

Carlo Patriarca and Carmelo Arizzi

Department of Pathology, Azienda Ospedaliera Melegnano, Via Pandina 2, 20070, Vizzolo Predabissi, Milan, Italy

Kidney cancer has the highest incidence in North America, Australia/New Zealand and Western and northern Europe. Each year in Europe about 45,000 deaths are caused by kidney cancer. Renal cell carcinoma (RCC) is the most common (80-85%) primary cancer of the kidney and 40% of patients with clinically localized RCC develop local recurrence or metastatic disease. Current research projects cover genetic and protein expression profile of various histological subtypes, as well as molecular profiling (prognostic and predictive) of individual tumors aimed for patient selection. Hence, to tailor the diagnosis for future tailoring of the treatment is one of the main goals. Moreover, the increase in detection of small (<4 cm) tumors incidentally identified in asymptomatic patients with a DSS of 95% raises the question of the real aggressiveness of small renal tumors. The main histotypes of RCC will be presented as well as the role of immunohistochemistry.

The anatomical prognostic factors encompass: 1) Tumor size: key component of the TNM staging system, remains one of the most important prognostic factors (4 cm: threshold for partial nephrectomy); 2) Perinephric/sinus fat involvement: portends a worse prognosis, but renal sinus fat involvement is associated with sarcomatoid differentiation and a higher risk of death; 3) Adrenal gland invasion: pT3a/M1: differentiate between direct invasion and metastatic deposit (prognostic difference not clear); 4) Venous tumor thrombus extention (RV or IVC): high risk for recurrent disease; 5) Lymph node involvement: portends a poor prognosis. N1-N2 subclassification remains controversial; 6) Presence of distant metastasis: portends a poor prognosis; 7) Number of metastatic sites, rather than actual location, dictates overall prognosis. Metachronous metastases are a favorable factor over synchronus metastases.

Among the histopathological prognostic factors are: 1) Nuclear grade; 2) Histological subtype; 3) Sarcomatoid differentiation; 4) Tumor necrosis; 5) Collecting system invasion; 6) Microvascular invasion. Weak points of each of these factors will be discussed briefly.

Molecular abnormalities in RCC involve proliferation, survival and tumour angiogenesis. Alterations in the *VHL* gene are the most common genetic abnormalities in RCC and lead to increased angiogenesis. The Raf/MEK/ERK pathway is activated by signalling through multiple cell surface receptors, leading to increased cell proliferation and survival. The phosphatidylinositol 3-kinase (PI3K) pathway includes sequential activation of several kinases and is also involved in signalling through many growth factor receptors. Activation of the PI3K pathway also leads to increased cell survival. Mutation of the *VHL* gene is a common genetic change that leads to RCC. Inactivation of *VHL* leads to dysregulation of the VEGF pathway. As a consequence of *VHL* gene mutation, the *VHL* gene product complex is disrupted and does not bind to HIF, allowing accumulation of HIF1-a and HIF2-a. This results in the transcription HIF-regulated genes, including those encoding VEGF, PDGF, transforming growth factor-alpha (TGF-a) and chemokine receptor 4. These factors act to induce angiogenesis, whose pathologic basis will be discussed, endothelial cell stabilisation, autocrine growth stimulation and organ-specific metastasis.

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NEW AVENUES FOR CANCER RESEARCH FROM PROTEIN NANOCRYSTALLOGRAPHY

Eugenia Pechkova

Nanoworld Institute University of Genova, Corso Europa 30, 16132 Genoa, Italy and Fondazione ELBA, Piazza SS. Apostoli 66, 00100 Rome, Italy

As a result of cooperation between the Nanoworld Institute and the European Synchrotron Radiation Facility in Grenoble, after the initial discovery of the atomic structure of human kinase CK2alpha (1-4) we have introduced new further developments in protein nanocrystallography, appearing to have profound potential impact in cancer research (5-9).

First of all, new details in protein crystal topography appear evident in conjuction also with AFM experimentations. The obtained images point to the existence of clear domains in the crystal 3D organization, quite pronounced and different in size and number between the classical protein crystals and the crystals grown by LB (Langmuir-Blodgett) protein nanotemplate. This result is furthermore in perfect accordance with that obtained by laser cutting of the corresponding protein crystals down to the nanosize and along the crystal domains. X-ray diffraction with highly focused synchrotron radiation down to 500 nm diameter strikingly provides unique and detailed atomic structure information in protein microcrystals down to the submicron size in several model systems, opening new avenues in protein crystallography.

With radiation damage being the most critical issue for protein structure determination under the intense synchrotron radiation, LB crystals were indeed confirmed as the most stable to radiation damage in a wide range of model systems. Crystals grown by nanotemplate still diffract at good resolution even after several steps of X-ray "burning", while the classic crystals decay very quickly at the same exposure. Finally due to this encouraging result, the LB method has also been successfully adapted to the EMBL advanced robotics system for protein crystallography and to the study of yet unsolved protein systems such as ribosomal proteins.

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MUC5AC EXPRESSION AND ITS PROGNOSTIC VALUE IN CHOLANGIOCELLULAR CARCINOMA

F. Pedica[1], L. Bortesi[1], P. Capelli[1], S. Pachera[2], A. Ruzzenente[2], A. Guglielmi[2], G. Martignoni[1], M. Chilosi[1], A. Scarpa[1] and F. Menestrina[1]

[1]Department of Pathology, University of Verona, Verona; [2]Department of Surgery and Gastroenterology, University of Verona Medical School, Verona, Italy

Cholangiocarcinoma (CC) is a malignant tumour composed of cells resembling those of the bile ducts and the second most common primary hepatic tumor after hepatocellular carcinoma, comprising 5-10% of primary liver neoplasms. Worldwide, cholangiocarcinoma accounts for 3% of all gastrointestinal cancer. Several studies have shown that the incidence and mortality rates of intrahepatic CC are rising, and those of extrahepatic cholangiocarcinoma are declining internationally (1). To date, radical surgery is the only therapy offering a potential cure for CC patients, whose prognosis is generally poor with survival limited to few months (2). At present, the lack of a sensitive and specific early diagnostic marker is an important reason why CC has a fairly late presentation. CC is classified according to its anatomic location into intrahepatic and extrahepatic, and, according to WHO classification, the term CC is used exclusively for carcinomas of intrahepatic origin, while tumors arising from the extrahepatic bile duct should be considered extrahepatic bile duct carcinomas (3).

Recently the Liver Cancer Study Group of Japan divided intrahepatic CC into three morphological types: mass-forming (MF), periductal infiltrating (PI) and intraductal growth (IG) (4). MF type is characterized by the presence of a spherical mass with a distinct border in the liver parenchyma, PI type presents tumor infiltration along the bile duct, occasionally involving the surrounding blood vessels and/or hepatic parenchyma, IG is characterized by papillary and/or granular growth into the bile duct lumen. PI type of CC presents a significantly higher frequence of perineural invasion, lymph node metastasis and extrahepatic recurrence than the MF type (5). The 5-year survival rates of patients with IG tumors or with MF tumors is significantly better than those of patients with MF plus PI tumors PI type alone (6).

Extrahepatic CC can further be subdivided into four types based on its location according to the Bismuth classification: type I, tumor involves the common hepatic duct distal to the biliary confluence; type II, tumor involves the biliary confluence; type IIIa, tumor involves the biliary confluence plus the right; hepatic duct; type IIIb, tumor involves the biliary confluence plus the left hepatic duct; type IV, multifocal or tumor involves the confluence and both the right and left hepatic ducts (7).

Although the entire biliary tree is at risk, tumors involving the bifurcation of the hepatic duct (Klatskin tumors) are the most common and account for 40% to 60% of all cases (8). Hilar cholangiocarcinoma as a specific entity was first described by Klatskin in 1965 and it has a PI type growth. Hilar invasive-type cholangiocarcinomas have been observed to often exhibit perineural invasion and nodal involvement. Moreover, extrahepatic CC display a sclerosing, nodular, and papillary phenotype of which the sclerosing or PI type is the most common.

Our aim was to find a sensitive and specific marker which could be detected in patient serum and be correlated with the tumor burden. Boonla C *et al*. (9) recently showed that MUC5AC mucin is present in significant concentrations in serum from patients with CC. MUC5AC is a secretory mucin normally expressed by the surface mucous cells of the human stomach and in the bronchial tract. In a recent report (10), MUC5AC resulted significantly correlated with neural invasion and advanced CC stage.

We investigated MUC5AC in intrahepatic CC and extrahepatic CC and found that it has a different expression depending on the type of growth in PI type, MF type and PI plus MF type. Our results suggest that MUC5AC is a specific prognostic marker and these three morphological type of CC have different immunophenotypes. Moreover MUC5AC is significantly correlated with a more aggressive pattern and with perineural invasion. MUC5AC is expressed in severe biliary epithelial dysplasia, so if it could be part of a carcinogenetic pathway should be investigated. Moreover, our results also suggest that both intrahepatic PI type and extrahepatic CC are probably the same pathology but arise in different locations. Further studies are needed to understand if this different expression may be an epiphenomenon of different carcinogenetic patterns between PI type and MF type of CC.

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EFFECT OF 1,25-DIHYDROXYVITAMIN D_3 ON DNA DAMAGE AND SURVIVAL OF PLATINUM-TREATED HUMAN CANCER CELLS

Fábio Pereira[1,2], María Jesús Larriba[1], Mercedes Herrera[3], María J. Muñoz-Alonso[1], Félix Bonilla[3] and Alberto Muñoz[1]

[1]Instituto de Investigaciones Biomédicas "Alberto Sols", Consejo Superior de Investigaciones Científicas - Universidad Autónoma de Madrid, Madrid, Spain;
[2]Faculty of Nutrition and Food Sciences of the University of Porto, Porto, Portugal;
[3]Hospital Universitario Puerta de Hierro, Madrid, Spain

Introduction: 1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) has antiproliferative, antiangiogenic and pro-differentiative effects in a broad range of cancer cells. In addition, $1,25(OH)_2D_3$ potentiates the effects of many antitumour agents. Platinum drugs are commonly used as anticancer agents. The development of new platinum-based agents and clinical combination studies has resulted in a growing interest of platinum-based cancer chemotherapy. These drugs act by the alkylation of DNA forming platinum-DNA adducts, leading to the interruption of essential cellular processes, and ultimately to cell death *via* activation of apoptotic pathways associated with DNA damage. *Aim and Methods:* The aim of this study was to investigate the influence of $1,25(OH)_2D_3$ on the sensitivity of human colon (SW480-ADH and HCT116) and lung (A549) cancer cells to oxaliplatin and cisplatin, which are respectively used in the clinic against these neoplasias. Global DNA damage was evaluated using the comet assay. The expression and localization of two DNA damage markers (H2AX and 53bp1) were studied by immunofluorescence. We performed cell proliferation and viability assays using different drug concentrations to calculate the IC_{50}. Known $1,25(OH)_2D_3$ target genes (E-cadherin, $p21^{CYP21}$, c-MYC) and two apoptosis markers (PARP and active caspase-3) were evaluated by Western Blotting (WB). We also performed flow cytometry analysis to study the putative effect of $1,25(OH)_2D_3$, oxaliplatin, and their combination on the cell cycle. *Results:* We observed that $1,25(OH)_2D_3$ moderately increased DNA damage caused by oxaliplatin (20 μM) in SW480-ADH cells (% tail DNA - vehicle = 4.6±1.7 *vs*. $1,25(OH)_2D_3$ = 3.0±1.2; $p<0.001$). According to the median-effect principle analysis of Chou and Talalay using CalcuSyn software (Biosoft, Ferguson, MO), $1,25(OH)_2D_3$ Pre-treatment slightly increased the sensitivity of SW480-ADH cells to oxaliplatin (IC_{50} vehicle *vs*. $1,25(OH)_2D_3$ = 1.3 *vs*. 0.8 μM) and of A549 cells to cisplatin (IC_{50}=8.9 *vs*. 4.4 μM). However, $1,25(OH)_2D_3$ increased the survival of SW480-ADH cells treated with oxaliplatin at concentrations of 20 and 30 μM (% cells = 13.4±1.4 *vs*. 21.4±1.5 and 10.8±0.3 *vs*. 15.9±0.8, respectively). No differences were observed for HCT116 cells. $1,25(OH)_2D_3$ did not change the expression and/or localization of the studied proteins by WB or immunofluorescence. In SW480-ADH cells, pre-treatment with $1,25(OH)_2D_3$ diminished apoptosis induced by oxaliplatin (10 and 20 μM), as observed by a reduction in the sub-G1 fraction in flow cytometry analysis (% cells = 10.5 *vs*. 2.7 and 12.8 *vs*. 7.2, respectively). *Conclusion:* $1,25(OH)_2D_3$ Treatment slightly increased global DNA damage and the sensitivity of SW480-ADH and A549 cells to oxaliplatin and cisplatin, respectively. However, at high oxaliplatin concentrations (10-30 μM) $1,25(OH)_2D_3$ increased survival of SW480-ADH cells. Altogether, these findings show that $1,25(OH)_2D_3$ may modulate the action of platinum anticancer drugs in human cancer cells, which merits further investigation.

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β-CATENIN, SURVIVIN AND CYCLIN-D1 EXPRESSION IN HUMAN HEPATOCELLULAR CARCINOMA

Stavros Peroukides, Alexandros Alexopoulos and Helen Papadaki

Department of Anatomy, School of Medicine, University of Patras, Rio, Patras, 26500, Greece

Introduction: β-Catenin is a downstream effector of the Wnt signalling pathway, regulating cell growth/survival. Activation of this pathway is caused mainly by mutations that stabilize β-catenin, allowing it to accumulate in the cytoplasm, then translocate to the nucleus and activate genes such as survivin and cyclin-D1. Survivin belongs to the inhibitors of apoptosis and current studies suggest its implication in both control of apoptosis and regulation of cell division. Cyclin-D1 is a regulator of G1/S-phase progression and its overexpression is linked to the development and progression of cancer. *Aim:* The investigation of the correlation between β-catenin, survivin and cyclin-D1 in human hepatocellular carcinoma (HCC). *Materials and Methods:* Immunohistochemical staining for β-catenin, survivin and cyclin-D1 was performed in 69 cases of HCC and adjacent normal liver tissue. *Results:* Normal liver showed diffuse membranous staining of β-catenin in hepatocytes. In contrast, cytoplasmic and nuclear accumulation of β-catenin was found in 42 (60.9%) and 33 (47.8%) out of 69 HCCs respectively. Loss of membranous β-catenin expression was observed in 53/69 (76.8%) HCCs and only 16/69 (23.1%) tumors showed a focal weak or moderate membranous staining. In normal liver, survivin and cyclin-D1 expression was absent. Nuclear survivin immunostaining was observed in 63/69 HCCs (91.3%) and nuclear cyclin-D1 expression in 46/69 (66.7%). There was no statistical correlation between β-catenin, survivin and cyclin-D1 expression. *Conclusion:* In HCC, overexpression of β-catenin, survivin and cyclin-D1 was detected. Activation of β-catenin was observed in our cases of HCC, while loss of membranous staining probably implies its role in tumor invasion. The nuclear localization of survivin and cyclin-D1 in tumor cells reflects their role in cell proliferation. In our study we failed to find an association between expression of β-catenin with survivin and cyclin-D1. The fact that overexpression of β-catenin did not correlate with survivin and cyclin-D1, probably suggests that another pathway works in human hepatocarcinogenesis.

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MULTIPLE CELLULAR MECHANISMS IN PROSTATE CANCER INVASION AND METASTASIS

Barbara Wegiel, Anders Bjartell, Johanna Tuomela, Nishtman Dizeyi, Martina Tinzl, Leszek Helczynski, Elise Nilsson, Leo E. Otterbein, Pirkko Härkönen and Jenny Liao Persson

Division of Experimental Cancer Research, Department of Laboratory Medicine, Clinical Research Center, Lund University, University Hospital, 205 02 Malmö, Sweden

Once prostate cancer becomes hormone refractory, cancer cells may rapidly gain the ability to invade and to metastasize to lymph nodes and distant organs. The progression through hormone-dependent to hormone-refractory and metastatic prostate cancer is poorly understood. Cyclin A1 is a cell cycle regulator that has been implicated in the progression of prostate cancer. We assessed protein and mRNA expression of cyclin A1, vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-2 and MMP-9 in primary malignant tumor and adjacent benign prostate tissue samples from 482 prostate cancer patients.

Prostate cancer samples had significantly higher cyclin A1 protein and mRNA expression than adjacent benign tissues. There was a significant correlation between expression of cyclin A1 and that of MMP-2, MMP-9, and VEGF, which have previously been found to influence cancer cell invasiveness. In addition, the effects of altered cyclin A1 expression in PC3 prostate cancer cells were studied *via* transient transfection and viral vector infection. Overexpression of cyclin A1 in PC3 prostate cancer cells was linked to increased invasiveness, whereas inhibition of cyclin A1 expression *via* short hairpin RNA expression led to a reduction of invasiveness. We further tested the impact of increased cyclin A1 expression in tumor invasion and metastasis in a mouse model of prostate cancer. We found that 80% of mice carrying PC3 cells overexpressing cyclin A1 had lymph node, liver, and lung infiltration, whereas all mice with tumors expressing control vector were free of liver and lung metastases and only one had lymph node metastases. In concert with androgen receptor, cyclin A1 increased VEGF and MMP2 promoter expression. Our results suggest that prostate cancer invasion is promoted by cyclin A1 *via* the alteration of the expression of specific signalling proteins and extracellular proteins. Furthermore, our findings that cyclin A1 regulates the expression of a growth factor signaling molecule (VEGF) and extracellular proteases (MMPs) and promotes tumor cell invasion and metastasis in part through MMPs and uPA(urokinase-type plasminogen activator) suggest that cyclin A1 may be a key regulator of tumor invasion and metastasis.

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DD3/PCA3 (DIFFERENTIAL DISPLAY CODE 3) IN PROSTATE CANCER DIAGNOSIS (EXPERIENCE FROM CZECH REPUBLIC)

M. Pesta, J. Klecka, L. Holubec, O. Topolcan, V. Eret, M. Chottova-Dvorakova, M. Babjuk, K. Novak, J. Stolz and M. Hora

University Teaching Hospital, Plzen and Charles University, Medical Faculty, Plzen, E. Benese 13, 305 99, Czech Republic

Introduction: Prostatic specific antigen (PSA) is very helpful in the early diagnosis of prostate cancer (Pca) but the main disadvantage is a low positive predictive value, which results in a high number of useless biopsies. For that reason we need new tests with better parameters. PCA3 is a prostate-specific non-coding mRNA that is highly over-expressed in prostate tumor cells. The aim of this study was to evaluate the diagnostic potential of PCA3 for PCa diagnosis. *Materials and Methods:* We examined altogether 199 patients. In the group of patients with suspicion of PCa we collected one tissue specimen core for PCA3 expression examination. According to the histologically verification 103 patients had benign prostatic hyperplasia (BPH), 12 patients prostatic intraepithelial neoplasia (PIN) and 84 patients prostate cancer. Total RNA was isolated and PCA3 and PSA expression quantified using Q RT PCR method. The PCA3/PSA mRNA ratio distribution was determined for both subject groups. The assess the ability of the PCA3 assay to predict biopsy outcome, the % biopsy positive was determined for different PCA3/PSA ratio ranges and PCA3 Ct. *Results:* In our study, the fraction of specimens yielding sufficient RNA for RT PCR analysis was only 75%. It was found that the levels of mRNA expression of PCA3 (Ct) were significantly higher ($p<0.045$) in patients with prostate cancer than in patients with benign prostatic hyperplasia. We found also statistically significant differences in the levels of mRNA expression of PCA3 (Ct) between patients with benign prostate cancer and patients with prostatic intraepithelial neoplasia (PCA3 Ct, $p<0.023$). *Conclusion:* The specificity of the PCA3 assay for prostate cancer seems to be useful for early detection of prostate cancer and also for differential diagnosis between patients with BPH and patients with prostate cancer.

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CALCIUM, VITAMIN D AND CANCER

Meinrad Peterlik[1], William B. Grant[2] and Heide S. Cross[1]

[1]Department of Pathophysiology, Medical University Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria; [2]Sunlight, Nutrition, and Health Research Center (SUNARC), P.O. Box 641603, San Francisco, CA 94164-1603, USA

A low vitamin D status and inadequate calcium intake are important risk factors for various types of cancer. Ecological studies using solar UV-B exposure as an index of vitamin D_3 photoproduction in the skin found a highly significant inverse association between UV-B and mortality in fifteen types of cancer. Of these, colon, rectal, breast, gastric, endometrial, renal and ovarian cancers exhibit a significant inverse relationship between incidence and oral intake of calcium. In addition, lung and endometrial cancer as well as multiple myeloma are considered calcium- and vitamin D-sensitive. Studies on tissue-specific expression of the *CYP27B1*-encoded 25-hdroxyvitamin D-1α-hydroxylase and of the extracellular calcium-sensing receptor (CaR) have led to an understanding of how locally produced 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) and extracellular Ca^{++} act jointly as key regulators of cellular proliferation, differentiation and function. Thus, impairment of anti-mitogenic, pro-apoptotic and pro-differentiating signaling from the 1,25-$(OH)_2D_3$-activated vitamin D receptor (VDR) and from the CaR in vitamin D and calcium insufficiency has been implicated in the pathogenesis of the aforementioned types of cancer. 1,25-$(OH)_2D_3$ and calcium interact in modulating cell growth in different ways: (i) Signaling pathways from the VDR and the CaR converge on the same downstream elements, *e.g.* of the canonical *Wnt* pathway; (ii) high extracellular calcium modulates extra-renal vitamin D metabolism in favor of higher local steady-state concentrations of 1,25$(OH)_2D_3$; (iii) 1,25$(OH)_2D_3$ may up-regulate expression of the CaR and thus augment CaR-mediated anti-proliferative responses to high extracellular Ca^{2+}. This can explain why combined supplementation is required for optimal chemoprevention of cancer by calcium and vitamin D.

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HAEMANGIOPOIETIC STEM CELLS AND TUMOR ANGIOGENESIS

S.O. Peters, T. Seifert, C. Huber, P. Dombach, P. Kuta, T. Wagner and S. Stoelting

Hämatologie/Onkologie, Universitätsklinikum S.-H.-Luebeck, Germany

Endothelial cell linings in the vessels of most tumors largely derive from non-malignant circulating progenitor cells. Autologous somatic endothelial precursors, procured by marrow aspiration or apheresis, may be used to introduce therapeutic principles into tumors.

We have studied endothelial development in cultured E14 murine embryonic stem cells (ECS), which provide a unique homogeneous cell system for studying early vasculogenic cell differentiation *in vitro*, and mapped the effects of vascular endothelial growth factor (VEGF) on these cells (Seifert T, Peters SO *et al*. Differentiation, 2008). After removal of leukemia inhibitory factor (LIF) a high proportion (36 percent) of undifferentiated state ESCs show positivity for endothelial CD31. This system allows for the describtion of characteristic endothelial differentiation patterns in embryoid bodies (EB) kept in culture for up to 30 days in differentiation culture medium, with or without supplemental VEGF. Directly after preculture and at day two in unsupplemented culture ELISA analysis showed no endogenous VEGF,

therafter VEGF levels rise. Early vasculogenic development and expression of selected genes were characterized using flow cytometry for specific antigens and quantitative RT-PCR. VEGF supplementation lead to qualitative changes in the EB vessels, specific activation of vasculogenesis-related genes (CD31, CD144 and ERG) and temporary downregulation of the VEGF receptor gene flk-1. VEGF supplementation did not produce measurable changes in the endothelial cell fractions as judged by surface antigen presence. This shows that that early ESCs may undergo endothelial differentiation through VEGF-independent pathways, whereas endothelial cell patterns in EBs are cytokine-dependent and fully stimulated by endogenous cytokine levels. Studies to characterize these pathways in embryonic and adult systems are underway. Translation of these results may enable us to manipulate adult heamangioblasts for tumor angiogenesis.

Another line of studies showed that JC and WEHI tumors transplanted into female mice displaying complete marrow chimerism, after receiving male bone marrow cells can serve as a murine experimental model to study manipulated and cultured endothelial precursors *in vitro* (Stoelting, Peters SO *et al*. Anticancer Research 2008). Using fluorescent *in situ* hybridization (FISH) analysis of adjacent cuts of the tumors, assigning CD31 and Y-chromosomes as markers gender origin of the perivascular endothelial cells can be determined. High proportions of male cells can be found in the perivascular endothelial cell linings of JC (60±4%) and WEHI (67±4%) tumors after implantation into normal male mice and in marrow chimeric female mice. This model allows for the study of *in vitro* treated marrow on tumorangiogenesis.

We also explore the potential of (PR3), which is responsible for a chronic vasculitis of small blood vessels in case of Wegener's Granulomatosis, to provoke inflammatory reactions within tumors. Others have shown that both overexpression of PR3 respectively it's subunit PR1 generate a neutrophilic inflammation In blood stem cells of patients suffering from chronic myeoloid leukemia. We isolated and introduced a pre-pro-form of PR3 into a pTracer™ -SV40 plasmid containing GFP. In ten experiments using human embryonic kidney cell line HEK-293, which was established from a human primary embryonal kidney transformed by adenovirus type 5 (Ad 5), using Nanofectin transfection kits (PAA) we observed transfection efficiency (rates) of approximately 85% after 14 days judged by fluorescence-microscopical registration of GFP-including cells. For transfections of CD 133 positive progenitor cells obtained after apheresis of peripheral blood stem cells mobilized after chemotherapy and G-CSF stimulation, we used the Amaxa Nucleofector device using an Amaxa programm. Here we observed GFP positivity in 60-70% of the viable cells after ten days in 3 different experiments. Analysis of the expression of plasmid-carried proteins is currently under way. We conclude that a pre-pro-form of PR3 may be transfected into HEK cells and CD133 positive precursors.

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ESTABLISHMENT AND CHARACTERIZATION OF THREE NOVEL CELL LINES DERIVED FROM HUMAN METASTATIC NEUROENDOCRINE TUMOR (NET)

Roswitha Pfragner[1], Annemarie Behmel[2], Harald Höger[3], Elisabeth Ingolic[4], Alfred Beham[5], Veronika Siegl[1], Oskar Haas[6] and Bruno Niederle[7]

[1]Department of Pathophysiology and Immunology, Medical University of Graz, Graz;
[2]Department of Human Genetics, Medical University of Graz, Graz;
[3]Core Unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, University of Vienna, Himberg;
[4]Research Institute for Electron Microscopy and Fine Structure Research, Technical University of Graz, Graz;
[5]Department of Pathology, Medical University of Graz, Graz;
[6]Medgen.at, Vienna;
[7]Department of Surgery, Medical University of Vienna, Vienna, Austria

Carcinoids are uncommon neoplasms derived from enterochromaffin (EC) cells of the neural crest. They have malignant potential and their incidence is steadily increasing. The only curative treatment option is surgery. We have focused on cultivation of these human neuroendocrine tumors (NET) as the most relevant models for the study of potential modes of therapy. Only few cell lines from human carcinoids have been established so far, among them our earlier established cell line, KRJ-I (1). The reason for the rare success in establishing carcinoid-cultures is due to the small amount of tissue available and the low mitotic activity in primary cultures. We have successfully established three continuously growing cell lines from tissue obtained from a metastatic human carcinoid of the terminal ileum: P-STS was derived from the primary tumor, L-STS from a lymph node metastasis, and H-STS from a liver metastasis. Immunocytochemical characterization proved the maintenance of characteristic neuroendocrine properties. The ultrastructure of the cells demonstrated the presence of neuroendocrine granules in the cytoplasm. Transplantation of the cell lines into SCID-mice proved their tumorigenicity. Cytogenetic analyses were done and mutation screening of P-STS excluded a *MEN1*-gene-associated genetic predisposition. Our data delineate the novel cell lines P-STS, L-STS and H-STS as neoplastic EC cell lines and demonstrate their utility as *in vitro*- and *in vivo*-models of small intestine carcinoids.

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GAL3BP REGULATES CELL MOTILITY AND INVASION IN BREAST CANCER CELLS

Enza Piccolo, Nicola Tinari, Maurizia D'Egidio, Daniela Semeraro, Albana Cumashi, Cosmo Rossi and Stefano Iacobelli

Department of Oncology and Neurosciences, and Foundation University "G. D'Annunzio", University "G. D'Annunzio", Chieti, Italy

Background: 90K/Mac-2 BP is a secreted glycoprotein originally identified in the supernatant of a breast cancer cell line (1). High serum and tumor tissue levels of the protein are associated with a shorter survival and a reduced response to chemotherapy in patients with different types of malignancy (2-6). Although the role of 90K/Mac-2 BP is not fully understood, it plays an important role in cell-cell and cell-extracellular matrix adhesive processes thanks to its binding to galectin1, -3 and -7 (2) and to several proteins of the extracellular matrix like collagens, fibronectin and nidogen (7). However, it is still not clear how the pro-adhesive features of 90K/Mac-2 BP affect tumor growth and progression. To address this issue, we generated and characterized a 90K/Mac-2 BP knocked-down clone of MDA-MB-231 human breast cancer cell line. *Methods and Results:* A stably 90K/Mac-2 BP silenced clone of MDA-MB-231 was obtained by siRNA. The level of expression of 90K/Mac-2 BP, evaluated at the mRNA and protein level by Real time PCReaction and ELISA respectively, was reduced by about 80% as compared to the mock transfected cells. As compared to controls, silenced cells showed no significant differences in terms of doubling time, indicating that 90K/Mac-2 BP is not involved in cell proliferation; on the other hand, silenced cells exhibited a reduced adhesiveness to fibronectin and collagenV, increased motility and increased matrigel invasiveness. Tumors derived from silenced cells xenografted in nude mice did not differ from controls in terms of growth rate, but produced a significantly higher number of lung metastases. Although preliminary, these data seems to indicate 90k/MAC-2 BP as an important molecule involved in tumor dissemination at least in breast cancer.

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SPECIAL ASPECTS OF SENTINEL NODE BIOPSY

Janusz Piekarski, Dariusz Nejc and Arkadiusz Jeziorski

Department of Surgical Oncology, Medical University of Lodz, Poland, Ul. Paderewskiego 4, 93-509 Lodz, Poland

Sentinel node biopsy (SNB) is a standard care in patients with breast cancer and patients with skin melanoma. Apart from use of SNB in our everyday clinical practice, in the Department of Surgical Oncology, Medical University of Lodz, Poland, numerous studies on special aspects of sentinel node biopsy procedure were conducted. In these studies, authors were focused mainly on (1) safety of medical staff, performing SNB; (2) possibility of the use of imprint touch cytology in assessment of SNB status in skin melanoma patients; (3) *ex vivo* use of SNB technique; and (4) the use of SNB technique in special clinical situations.

In order to assess the safety of medical staff performing SNB in patients with breast cancer and in patients with skin melanoma, authors measured the absorbed doses of radiation to the hands of medical staff. During lymphoscintigraphy and during surgical procedure on different parts of hands of medical staff, a total of 57 highly sensitive thermoluminescent dosimeters were placed. Altogether, 2065 measurements were performed during 35 procedures. The results revealed that the maximum recorded dose during the study was 1900 times smaller than the current one-year dose limit recommended by the International Commission on Radiological Protection.

To assess whether the reliability of imprint touch cytology

(ITC) of sentinel nodes in skin melanoma patients allows intraoperative decisions to be made regarding simultaneous radical lymphadenectomy we performed a study on 148 sentinel nodes removed from 85 skin melanoma patients. We found that ITC of sentinel nodes is a reliable method. The risk of overtreatment due to false-positive results of ITC of sentinel node was absent in our study.

We performed *ex vivo* blue-dye SN mapping in postmastectomy specimens to assess whether the main lymphatic tract leading from periareolar plexus to SN is completely removed during mastectomy. We assumed that *ex vivo* identification of SN may be possible only if entire lymphatic tract leading to sentinel node is removed within the postmastectomy specimen. Our experiment revealed that the use of transverse skin incision during modified radical mastectomy may not be a best choice for breast cancer patients; when the transverse incision was used, we were able to identify sentinel nodes only in 31% of cases.

We considered the use of SNB in a patient with five synchronous primary skin melanomas, a special clinical situation. The patient underwent excisions of all five primary tumors along with five simultaneous sentinel node biopsies. We successfully identified lymph flow from all skin areas to three different lymph node basins and successfully retrieved six sentinel nodes from these basins. In our opinion, this case illustrates that performing multiple sentinel node biopsies in patients with multiple primary skin melanoma is possible.

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BRAIN METASTASES IN OVARIAN CANCER: OVERVIEW AND OPTIMAL TREATMENT

Klaus Pietzner, Khalid El Khalfaoui, Gülten Oskay-Özcelik, Dirk Boehmer, Werner Lichtenegger and Jalid Sehouli

Department of Gynecology and Obstetrics, Division of Gynecologic-Oncology, Charité-Campus Virchow Klinikum, University Medicine of Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

Ovarian cancer is one of the leading causes of mortality in the field of gynecologic oncology. Central nervous system (CNS) involvement however is rare in presentation and seems to be associated with a very poor prognosis. Clinical as well as autopsy studies in the last decades have confirmed the rarity of occurrence of brain metastases in ovarian cancer, but several authors have recently observed a sharp rise in incidence. While most authors attribute this increase of CNS involvement to prolonged survival achieved through advances in chemotherapy and surgical management, others see it resulting from improved imaging or chemotherapeutic impairment of the blood-brain barrier. Brain metastases from ovarian cancer can present with a panel of often unspecific symptoms which usually results in a late diagnosis of CNS relapse, since cerebral imaging is not part of the routine follow-up. Even serum-CA-125 levels, a valuable tool in predicting recurrence of distant disease, was shown to be incapable of reliable detection in regard to metastatic brain manifestation, leaving the clinician with the need for close patient observation for neurological symptoms in order to diagnose brain metastasis at an early stage. While some reports only indicate the presence of extracranial disease at CNS relapse and time from diagnosis of ovarian cancer to development of brain metastases as prognostic factors for survival, other studies demonstrate the negative impact of multiple cerebral lesions on survival, when compared to single brain metastases. Though great efforts have been made to develop multimodal therapeutic strategies to challenge the rising incidence of brain metastasis in ovarian cancer, CNS involvement is still related with a very poor prognosis. It was shown that a multi-modal approach, combining surgical resection with radiation therapy and even chemotherapy promises the best prolongation of survival and did result in long-term remissions in a few cases. But this aggressive strategy is not applicable to all patients, often due to overall status or inaccessibility of brain metastases to the neurosurgical approach. In these cases stereotactic radiation therapy or gamma-knife-surgery is recommended by many authors to remove single metastatic brain lesions. These techniques should be further discussed as an alternative for whole-brain radiation therapy, as several studies expand its use to other indications such as multiple lesions and with promising results. Based on our large multicenter study including 73 patients with brain metastases from ovarian cancer, we will discuss predictive and prognostic factors as well the current best treatment.

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EXPLOITATION OF THE BIOLOGICAL FEATURES OF BRAIN TUMOURS IN PURSUIT OF NEW THERAPEUTIC STRATEGIES: *IN VITRO* STUDIES

Geoffrey J. Pilkington, Samantha A. Murray, Samantha Higgins, Kathryn Fry, Laura Donovan, Suzanne Birks, Zaynah Maherally and Naif Alsiweheri

Cellular and Molecular Neuro-oncology Group, Cellular and Molecular Medicine Research Division, Institute of Biomedical and Biomolecular Sciences, School of Pharmacy and Biomedical Sciences, University of Portsmouth, St Michael's Building, White Swan Road, Portsmouth PO1 2DT, UK

Primary, malignant brain tumours are among the most therapeutically-resistant of all cancer. This resistance is a consequence of the unique biological properties which characterise them. The blood–brain barrier (B-BB) poses a specific obstacle to chemotherapy, while neoplastic glial cells are particularly efficient at repairing DNA damage breaks

caused by both chemo- and radiotherapy. In addition, the ability of glioma cells to diffusely invade the contiguous normal brain adds to this resistance. During invasion, tumour cells transiently arrest from the cell cycle, rendering them refractory to radiotherapy. Moreover, while certain cytotoxic drugs can reach the major tumour mass by virtue of disruptions of the B-BB, cells which have invaded deep into the brain are protected from them since they are invested in regions of intact B-BB. Tumour resistance is also thought to be facilitated by the existence of small populations of self-renewing stem cell-like cancer cells (SC-LCS) within glial tumours. There is increasing evidence that this population of cells are also highly migratory and thus may contribute to both resistance and local dissemination. To contribute to the complexity surrounding neurological tumours, in addition to primary brain tumours, the brain is a fertile site for the growth of secondary tumours, indeed up to a quarter of all tumours will spread to the central nervous system at some stage.

In order to study this complex biological picture we have established – from human cells and under human serum supplementation conditions – three-dimensional live cell model systems of both tumour invasion and the B-BB (for studies on cancer cell metastasis to the brain). We have also segregated CD133-positive SC-LCSs by use of AutoMACS immunobead separation from early passage, biopsy-derived glioblastoma and studied their complex behaviour, lineage properties and response to microenvironmental factors. We have used these cells and the above models to investigate four putative therapeutic targets: CD155 (poliovirus receptor), CD44 (hyaluronic acid receptor), GD3 (cell surface disialoganglioside 3) and NG2 (neuronal-glia2 chondroitin sulphate proteoglycan), which may provide a means of either selectively killing tumour cells or inhibiting their invasive properties. We have also examined the tumour cell mitochondrion as a possible target for enhanced therapeutic effect by way of tricyclic drug mediated apoptosis. We believe that each of these approaches may carry potential for future clinical studies.

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EPIDEMIOLOGY OF VITAMIN D DEFICIENCY AND CANCER MORTALITY

Stefan Pilz

Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, Austria

There is growing evidence that vitamin D exerts anticarcinogenic effects. Ultraviolet-B (UV-B) radiation, which is required for vitamin D production in the skin, was found to be inversely associated with cancer incidence and mortality. Recent studies have largely but not consistently shown that low 25-hydroxyvitamin D [25(OH)D] levels, which are considered to be the best indicator of vitamin D status, are a significant risk factor for cancer mortality. Circulating 25(OH)D levels were also associated with improved overall survival in colorectal and lung cancer patients and vitamin D deficiency was observed in patients with autoimmune, infectious and cardiovascular diseases. Significant seasonal variations in 25(OH)D levels and the association of vitamin D deficiency with reduced physical activity are, however, possible sources of confounding in epidemiological studies. Randomized controlled trials are therefore urgently needed to evaluate whether vitamin D supplementation reduces cancer incidence and mortality. The optimal 25(OH)D levels for human health, that should be achieved by vitamin D supplementation, still remain to be elucidated but there exists a wide consensus that every adult should have 25(OH)D levels of at least 30 ng/ml.

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CHARACTERIZATION OF NON-SMALL CELL LUNG CANCER USING TILING RESOLUTION BACTERIAL ARTIFICIAL CHROMOSOME MICROARRAYS

Sofi Isaksson[1], Annette Salomonsson[1], Pär-Ola Bendahl[1], Linda Ivarsson[1], Leif Johansson[2], Per Jönsson[3], Anna Karlsson[1], Maria Soller[4], Göran Jönsson[1] and Maria Planck[1]

Departments of [1]Oncology, [2]Pathology, [3]Thoracic Surgery and [4]Clinical Genetics at Lund University Hospital, Sweden

Lung cancer is the leading cause of cancer death in the world and, although diagnostics and therapeutics have improved during the past two decades, the overall 5-year survival rate is still below 15%. Eighty percent of all lung cancer is of the non-small cell type (NSCLC) and of these, about one fourth are stage I-IIIA tumors and thus accessible for surgery. The 5-year survival after successful resection is approximately 50%, but the outcome is heterogeneous, even within the same clinicopathological stage.

Development of new techniques in molecular biology, such as efficient characterization of tumors at the gene level with subsequent correlations to diagnostics and prognostics, is therefore of great importance. However, our knowledge of genomic imbalances in lung cancer and the oncogenic consequences of these alterations are still limited.

The use of whole-genome tiling resolution bacterial artificial chromosome (BAC) microarrays allows for characterization of DNA copy number changes at a resolution only limited by the number of BAC clones used for the arrays. In the present study, 32,433 overlapping BAC clones covering the whole genome were used, implying that the tumor DNA could be analyzed with an average resolution of 70 kbp. This

is, to our knowledge, the first study using this approach on clinical specimens of primary lung cancer. Freshly frozen biopsies of primary NSCLCs were obtained from patients operated in 1989-2003 at the Lund University Hospital, Sweden. In all, 62 early stage primary NSCLCs (48 adenocarcinomas and 14 squamous cell carcinomas) were profiled to allow for subclassification, identification of recurrent changes, and search for candidate genes. Alterations were observed in all chromosomes and the tumors displayed complex DNA copy number profiles with numerous gains and losses. Frequent amplifications and homozygous deletions were observed in regions harboring oncogenes and tumor suppressor genes, respectively. Thus, the use of whole-genome tiling resolution BAC microarrays is applicable for identification of novel candidate genes in lung cancer development.

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GAMMA/DELTA (γδ) T-CELL RECEPTOR (TCR)+ T CELLS IN EPITHELIAL OVARIAN CARCINOMA (EOC): CLONAL EXPANSIONS, TUMOR CELL LYSIS AND POTENTIAL FOR γδ TCR-BASED IMMUNOTHERAPY

C.D. Platsoucas[1,2], W.-J. Jung[1], F. Whitfield[1], A. Tsygankov[1], E. Hernadez[3], R.S. Freedman[4] and E.L. Oleszak[1,2]

[1]Department of Microbiology and Immunology and [3]Department of Obstetrics and Gynecology, Temple University School of Medicine, Philadelphia, PA; [2]Center for Molecular Medicine and Department of Biological Sciences, College of Sciences, Old Dominion University, Norfolk, VA and [4]Department of Gynecologic Oncology, M.D. Anderson Cancer Center, Houston, TX, USA

Alpha/beta TCR+ T-cells recognize primarily peptides in association with self-MHC, whereas, most Vδ1+ T-cells recognize whole proteins, and most Vδ2+ T-cells recognize phosphoantigens, lipids, and other ligands, both in an MHC-independent manner. We examined whether clonally expanded γδ TCR+ T-cells are present in tumor infiltrating lymphocytes (TIL) and PBMC from patients with EOC. VγI, VγII, Vδ1 and Vδ2 TCR transcripts were amplified from TIL and PBMC by two sided V region subgroup specific PCR, followed by cloning and sequencing. Sequencing analysis revealed the presence of substantial proportions of identical copies of VγI (in 8 of 12 patients; 67%), VγII (in 9 of 11 patients; 82%), Vδ1 (in 6 of 6 patients; 100%), and Vδ2 (in 4 of 7 patients; 57%) TCR transcripts in TIL from patients with EOC. γ- and δ-chain TCR transcripts were also clonally expanded in the PBMC from patients with EOC (in all 3 patients examined). In certain patients identical TCR transcripts were clonally expanded in both PBMC and TIL from the same patient. These γ- and δ-chain TCR clonal expansions were very strong and statistically significant. T-cells are comprised of a very large number of different T-cell clones, each expressing a different TCR. Because of their large numbers, the probability of finding by chance substantial proportions of individual TCR transcripts in an independent sample of T-cells is negligible. The appearance of multiple identical copies of TCR transcripts must be the result of specific antigen-driven proliferation and clonal expansion of individual T-cell clones. Full length copies of clonally expanded γ- and δ-chain TCR transcripts were constructed by PCR-mediated four-segment ligation and appropriate combinations were expressed into TCR beta-chain negative mutant Jurkat T-cells (which stain positive with anti-granzyme B antibody) using a retroviral expression system. Expression of the γδ TCR was determined using an anti-γδ TCR mab. These transduced Jurkat T-cells expressing the clonally expanded γ- and δ-chain TCR transcripts: (i) induced apoptosis *via* caspase-9 activation on ovarian tumor cells; and (ii) exhibited strong cytolytic activity against ovarian tumor cells. These results provide the basis for the development of new approaches for γδ TCR+ T-cell immunotherapy.

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COMBINATION IMMUNOTHERAPY: NEUTRALIZATION OF THE TUMOR ESCAPE AND IMMUNOSUPPRESSIVE MECHANISMS MAY BE REQUIRED FOR CLINICALLY EFFECTIVE TUMOR VACCINES AND ADOPTIVE IMMUNOTHERAPY APPROACHES (AN OVERVIEW)

Chris D. Platsoucas

Center for Molecular Medicine and Department of Biological Sciences, College of Sciences, Old Dominion University, Norfolk, VA, USA

Tumor cells have been very appropriately called masters in disguise and deceit from detection and destruction by the cells of the immune system. Tumors employ a variety of mechanisms to achieve this purpose. These mechanisms are collectively designated as tumor escape mechanisms and have been shown to be active in patients with a variety of cancers. Although substantial progress has been made in identifying tumor antigens, in determining their molecular structure and characteristics and in developing tumor vaccines, they have been so far in general ineffective for the treatment of patients with cancer. These cancer vaccines induced biological responses in as many as 50% of the patients, but objective clinical responses in less than 4%. In contrast to cancer vaccines, adoptive immunotherapy approaches, which involve the transfer to the host of large numbers of *in vitro* grown activated autologous T-cells able to destroy the tumor, have been shown to be effective and induce objective clinical responses in 50-70% of patients with metastatic melanoma.

The effectiveness of both cancer vaccines and adoptive immunotherapy approaches is substantially hindered by tumor escape mechanisms, which include: (i) increased numbers and proportions of CD4+CD25+ immunoregulatory suppressor T-cells (Tregs) at the tumor site and the peripheral blood. Reduction of the Tregs in patients with cancer using ONTAK resulted in significantly increased responses to tumor vaccines. Extensive lymphodepletion is required for effective adoptive immunotherapy approaches, and may involve reduction of Treg, and other tumor escape mechanisms (see below); (ii) increased numbers and proportions of tumor-associated macrophages/monocytes; (iii) production of immunosuppressive factors, such as TGF-beta and IL-10, by tumor cells and mononuclear cells (non-malignant cells) infiltrating the tumor or present in the peripheral blood; (iv) increased expression of cell surface molecules (PD-1 and CTLA-4) inhibitory of the effector function of tumor infiltrating lymphocytes; (v) lack of co-stimulatory molecules on tumor cells; (vi) downregulation of HLA class I expression on the surface of the tumor cells. We believe that the simultaneous neutralization of several of these complex and highly effective tumor escape mechanisms is very likely required for the development of clinically effective cancer vaccines and for increasing the effectiveness of adoptive immunotherapy approaches.

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THE PROMYELOCYTIC LEUKEMIA PROTEIN: FROM ANTIVIRAL RESPONSE TO CANCER

Pavlina Plevova[1,2], Ivana Jeziskova[1], Sylwia Walczyskova[1], Anna Krepelova[3], Kristyna Pavlikova[3], Alena Puchmajerova[3], Jana Zapletalova[4] and Eva Silhanova[1]

[1]Department of Medical Genetics, Faculty Hospital of Ostrava, Tr. 17. listopadu 1790, 708 52 Ostrava 8; [2]Institute of Pathology and Laboratory of Molecular Pathology, Medical Faculty, Palacky University, Hnevotinska 3, 775 15 Olomouc; [3]Institute of Biology and Genetics, 2nd Medical Faculty of Charles University, V Úvalu 84, 150 00 Prague-Motol; [4]Institute of Biophysics, Palacky University, Hnevotinska 3, 775 15 Olomouc, Czech Republic

The *PML* (promyelocytic leukemia) gene encodes a multifunctional protein involved in antiviral response, transcription control, induction of apoptosis, growth arrest, cellular senescence, and DNA damage repair. We have found down-regulated PML protein expression in about one fifth of colon cancer and one third of breast cancer tissues. Breast cancer tissues from germline *BRCA1* gene mutation carriers rarely down-regulate the PML protein, suggesting a functional relationship between the BRCA1 and PML proteins. There are several proteins both bound in PML nuclear domains and involved in the BRCA pathway activated in response to double-strand DNA damage. This also suggests there might be functional association between these systems. This could explain why breakage of some proteins of the BRCA pathway leads to an increased sensitivity to infections and also other clinical aspects. We are going to present a hypothesis on the essence of potential cooperation between PML nuclear domains and the BRCA pathway and its possible biological, clinical and evolutionary consequences.

We have studied the *PML* gene in patients with hereditary or familial breast and colon cancer, colon polyposis and stomach cancer in order to test a hypothesis that germline *PML* gene mutations might predispose to cancer. We found a single nucleotide substitution in the alternatively spliced exon 7b, c.1710+1355G>C (p.A570+232>P570+232) in about one third of patients with colon cancer and/or colon polyposis and the majority of patients with gastric cancer (the results were statistically significant). We suggest a new hypothesis on the pathogenesis of these tumors.

In conclusion, it is highly probable that an important interplay exists between PML nuclear domains and the BRCA pathway. Germline carriers of the c.1710+1355G>C substitution in the alternatively spliced exon 7b of the *PML* gene are at an increased risk of gastrointestinal polyposis and cancer.

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HETEROGENEITY OF HEMOGLOBIN IN BLOOD IN THE MODEL OF TUMOR GROWTH

Tatiana Polishko and Natalia Shtemenko

Dnipropetrovs'k National University, 13 Naukoviy by-street, 49050 Dnipropetrovs'k, Ukraine

The energy metabolism of tumor cells is quite different from that of normal cells. A characteristic property of malignant cells is their high rate of glycolysis. Normal cells depend on oxidative phosphorylation to synthesize ATP, but even in the presence of oxygen, cancer cells exhibit an increased capacity for lactate production. The enhanced rate of aerobic glycolysis correlates in general with the degree of malignancy. Although the production of ATP *via* aerobic glycolysis is inefficient, this selective adaptation may be a mechanism of survival for tumor cells under conditions of poor vascularization.

Normal adult human red blood cells (RBC) generate energy almost exclusively through the metabolism of glucose primarily *via* the Embden-Meyerhof pathway and the pentose phosphate shunt. These pathways produce the cellular energy crucial to RBC survival and maintenance of proper cell function. Malignant cells show an increased glucose uptake *in vitro* and *in vivo*. This process is thought to be mediated by Gluts, the human erythrocyte glucose transporter, the

expression and activity of which is regulated by oncogenes and growth factors. In our previous work, we have described the new Re-Pt antitumor system, where cisplatin and cluster rhenium compounds were used against Guerin's carcinoma development in rats. In this work, we present Glu levels and some types of Hb in blood under influence of the Re-Pt system with the use of three rhenium substances.

The following Cluster rhenium compounds with organic ligands were investigated: Re1 $[Re_2(i\text{-}C_3H_7CO_2)_4Cl_2]$; Re2 cis-$[Re_2(AdCOO)_2Cl_4].2CH_3CN$; Re3 $[Re_2(GABA)_2Cl_5(H_2O)]2H_2O$. Wistar rats were inoculated with tumor Guerin's carcinoma (T8) cells. A single intraperitoneal administration of cisplatin at the dose of 8 mg/kg was made on 9 day after the tumor inoculation. The intraperitoneal administration of the Re1-3 at the dose of 7 μM/kg in liposome forms began on day 3 after the inoculation of the tumor cells and was repeated every 2 days until day 21. Concentrations of red blood cells and their morphological forms were measured. Fetal Hb (FHb) and glycosylated Hb (HbA1c) were assayed by column liquid chromatography.

Investigations of the Hb heterogeneity showed that in the blood of animals of T8 + cisplatin group the concentration of HbA1c was much higher (up to 7%) than in the control group (4.2-4.5%). In some cases, where the inhibition of the tumor by cisplatin was not so strong, we observed the presence of FHb (0.3%), which was almost absent in the other groups. Higher level of HbAc1 is known to appear in RBC and plasma of the patients with some types of cancer. FHb is an established serological indicator of cancer. Cluster rhenium compounds may influence the energy metabolism of RBC and the process of Hb forms expression. Further investigation of Hb heterogeneity in this model may lead to propositions of markers for the effectiveness of anticancer therapy.

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GAMMA-GLUTAMYLTRANSFERASE OF CANCER CELLS AT THE CROSSROADS OF REDOX REGULATION, TUMOR PROGRESSION, DRUG RESISTANCE AND DRUG TARGETING

A. Corti[1], T.L. Duarte[2], A. Paolicchi[1], S. Dominici[1], G.D.D. Jones[2], P. Dilda[3], P.J. Hogg[3] and A. Pompella[1]

[1]Department of Experimental Pathology, University of Pisa, Italy;
[2]Radiation and Oxidative Stress Group, Department of Cancer Studies and Molecular Medicine, University of Leicester, UK;
[3]UNSW Cancer Research Centre, University of New South Wales, Sydney, Australia

Gamma-glutamyltransferase (GGT) is often significantly increased in human malignancies and its role in tumor progression, invasion and drug resistance has been repeatedly suggested (Pompella *et al*: Curr Opin Pharmacol *7*: 360, 2007). Previous studies have repeatedly documented a role of GGT in cellular redox balance, through the production of a low but persistent oxidative stress (see *e.g.* Paolicchi *et al*: Biochem. Pharmacol *64*: 1029, 2002). When GGT-overespressing cells were incubated in the presence of GGT substrates and a source of catalytic iron, increased levels of DNA damage were observed (Comet assay). This phenomenon was suppressed by specific GGT inhibitors such as ABBA, as well as by iron chelator DFO and antioxidants BHT and Trolox C. Interestingly, higher levels of basal DNA damage were observed in GGT-overexpressing cells as compared to low-expressing ones. These results suggest thus a role of GGT expression *per se* in tumor progression.

On the other hand, GGT has been implicated in cancer drug resistance as it participates in the reconstitution of cellular glutathione and related antioxidant/antitoxic defences. However, more complex effects of GGT expression can take place, both intra- and extracellularly. In fact the enzyme can play as a factor both in drug resistance and drug sensitivity, as documented by the results of our latest studies: i) The protective effects of GGT against cisplatin cytotoxicity are independent of intracellular glutathione, and depend rather on extracellular reactions of cisplatin with GGT-derived thiol metabolites, leading to formation of adducts which are far less cell permeable (Franzini *et al*: Eur J Cancer *42*: 2623, 2006); ii) On the contrary, in the case of 4-[*N*-(*S*-glutathionyl-acetyl)amino] phenylarsinous acid (GSAO) – a promising anti-angiogenic drug – cell membrane GGT activity acts as a sensitizing factor. The gamma-glutamyl residue of GSAO is in fact cleaved at the surface of GGT-expressing cells, thus producing the metabolite GCAO. The latter is transported across the plasma membrane, and eventually reacts with its mitochondrial targets (Dilda *et al*: 2008, manuscript submitted). This information can also explain GSAO kidney toxicity at high doses: GGT is in fact expressed at high levels in tubular epithelia; iii) Recent observations highlight NO and NO-donor agents (*e.g.* *S*-nitroso-glutathione, GSNO) as chemosensitizing agents, capable of potentiating the action of several anticancer drugs (Sullivan *et al*: Curr Pharm Des *14*: 1113, 2008). As GGT possesses the selective ability to metabolize GSNO – thus releasing its NO load – its expression may well be exploited to target NO to GGT-expressing tumor cells. We have investigated the kinetics of GGT with respect to GSNO using our innovative fluorimetric method® based on copper decomposition of nitrosothiol metabolites and reaction of released NO with 4,5-diaminofluorescein (Angeli *et al*: Arch Biochem Biophys 2008, in press). The results indicate a K_m of GGT for GSNO of ~0.4 mM, comparable with the K_m value for glutathione, which confirms the feasibility of using GSNO as an efficient pro-drug in order to perform selective NO treatment of GGT-

expressing tumors. Future studies will substantiate the applicability and usefulness of such an approach to therapy.

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VITAMIN D AND CANCER: AN OVERVIEW

Alina Carmen Porojnicu[1], Zoya Lagunova[1], Arne Dahlback[2] and Johan Moan[1,2]

[1]Department of Radiation Biology, Rikshospitalet - Radiumhospitalet HF, Oslo;
[2]Department of Physics, Oslo University, Norway

After Garland and Garland in 1980 reported that the mortality of colon cancer increases with increasing latitude and hypothesized that this may be explained by induction of vitamin D by solar radiation, a large number of studies have been devoted to this association. Multiple approaches have been chosen, from ecological studies to randomized clinical trials. Eventhough some studies have shown no correlation, most of them support the concept that a good vitamin D status is anticarcinogenic by either reducing the risk of developing cancer or preventing cancer progression. Our main finding is that prognosis of a number of cancer forms in Norway is dependent on season of diagnosis. Generally, diagnosis in summer and autumn leads to the best prognosis. Most likely, this can be explained by the higher concentrations of vitamin D we observe in serum in summer and autumn than winter and spring. Solar ultraviolet radiation, UV, is believed to contribute by up to 90% of the circulating levels of vitamin D, the rest being obtained from diet. Vitamin D, activated through hydroxylations, has anti-cancer properties. Some of the molecular and genetic mechanisms behind its biological functions are known, while some remain unclear. The annual previtamin D photosynthesis is larger in the southern part of the country than in the northern part. Due to climatic differences, the real sun exposure of people can be different from the ambient ones. We investigated this by determining the incidence rates of squamous cell carcinoma, which are related to the real UV exposures. These are a factor of about three larger in south than in north. Unexpectedly, there were no significant differences in survival for different latitudes and annual UV exposures. This may be due to the 20% higher vitamin D intake in North Norway than in South Norway. We found that over a larger range of latitudes (from Scandinavia to Australia), the ratio of death rates to incidence rates (a crude measure of survival) decreases with decreasing latitude. The epidemiological evidence together with the biological knowledge of the anticancer effects of vitamin D should prompt more studies aimed at defining an optimal vitamin D status and the safest ways to achieve this.

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CHROMOGRANIN A

M. Prazakova, O. Topolcan, L. Holubec, J. Vrzalova, M. Pesek, M. Casova and J. Safranek

Faculty Hospital and Medical School in Pilsen, E. Benese 13, 305 99 Plzen, Czech Republic

Aim: To find out the rate of CgA serum level positivity in patiens with lung cancer, colorectal cancer and prostate cancer. *Materials and Methods:* Serum levels of CgA were assessed using immunoradiometric analysis (IRMA) with commercially available assay kit from Schering – CIS BioInternational (France). Groups of patients: i) Control group – 57 patients with no history and no evidence of cancer disease at the time of serum examination; ii) Patients with malignant disease of the lung (I-III stage) – 90 patients at the time of primary diagnosis (prior to any treatment) 112 patients with non small cell lung cancer (NSCLC) during follow-up; iii) Patients with carcinoid tumor – 18 patients; iv) Patients with prostate cancer; v) Patients with non-tumor disease – renal failure, chronic liver failure. *Results:* The group of patients with lung cancer had significantly higher serum levels of CgA in comparison to the control group (p 0,001). Serum values of CgA in patients with progression were significantly higher than those in patients with remission and in the control group, we did not observe any statistically significant difference between the control group and the group of patients in complete remission. During the follow-up period, remission values were significantly different from levels examined at the time of primary diagnosis (p 0.001) and from the values of CgA in patients with progressive disease (p 0.001). CgA serum levels were elevated above cut-off levels in 56% of patients with SCLC, 43% of patients with non-small cell lung cancer and in more than 80% of patients with carcinoid tumors. CgA serum level changes correlated with therapy effect, as demonstrated in several case reports. False higher values of CgA could be seen in patients with chronical liver failure and renal failure. It appears that chromogranin values correlate with prognosis in the case of prostate cancer, colorectal cancer and breast cancer. *Conclusion:* Chromogranin A serum levels could be used in the diagnosis of lung cancer of a neuroendocrine character. Based on this study, CgA seems to be a helpful parameter for the follow-up and therapy monitoring of these diseases.

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INHIBITION OF TOPOISOMERASE I BY ERLOTINIB IN CANCER CELL LINES: EFFICACY OF COMBINED TREATMENTS WITH ERLOTINIB AND TOPOISOMERASE INHIBITOR

Refael Peleg[1], Dmitri Bobilev[2] and Esther Priel[1]

[1]The Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences, Ben Gurion University of the Negev;
[2]Department of Oncology, the Soroka Hospital Medical Center, Beer Sheva, 84105, Israel

Erlotinib is a tyrosine kinase inhibitor used to target the epidermal growth factor receptor (EGFR) and is currently in clinical use as an anticancer drug. We previously showed that certain tyrosine kinase antagonists, tyrphostins, inhibited the catalytic activity of cellular topoisomerase I (topo I). Therefore, it may be assumed that erlotinib exerts its anticancer activity by also inhibiting topo I. Purified and nuclear extract-derived topo I were added to topo I reaction mixture in the presence or absence of erlotinib (10^{-4}-10^{-12} M). Here we show that erlotinib inhibited the DNA relaxation activity of purified topo I as well as topo I derived from breast cancer (MCF7) and prostate cancer (PC3) cell lines.

To examine the effect of erlotinib on the cellular topo I, MCF7 and PC3 cells were treated with different doses of erlotinib and the effect on topo I was determined. Erlotinib treatment significantly reduced the cellular DNA relaxation activity of topo I but did not affect the level of topo I protein. Examination of the mode of action revealed that erlotinib reduced the DNA-binding ability of topo I. The MCF7 cells demonstrated a relatively high resistance to treatment with erlotinib, and with camptothecin (CPT), a topo I inhibitor, administered separately. A combined treatment based on erlotinib and CPT increased the anticancer effect of CPT as well as the CPT-mediated inhibition of topo I activity.

The results of this study show that topo I is a novel target of erlotinib and suggest that a combination of erlotinib with topo I inhibitors may demonstrate an effective anticancer treatment.

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IMMUNOSUPPRESSIVE DRUG FTY720 SENSITIZES PROSTATE CANCER CELLS TO RADIOTHERAPY BY INHIBITION OF SPHINGOSINE KINASE-1

Dmitry Pshezhetskiy[1], Torsten Boehler[2], Nicolas Doumerc[3], Muriel Golzio[4], Justin Tessie[4], Volker Brinkmann[5], Bernard Malavaud[3,4], Jonathan Waxman[1] and Olivier Cuvillier[4]

[1]Department of Oncology, Imperial College London, London, UK;
[2]U466, INSERM, Toulouse, France;
[3]Department of Urology, Rangueil Hospital, Toulouse, France;
[4]Department of therapeutic targets, IPBS, Toulouse, France
[5]Novartis Pharma, Basel, Switzerland

A sphingolipid analogue, FTY720, is a novel immuno-suppressive drug – an agonist of sphingosine 1-phosphate (S1P) receptors. Recently we identified sphingosine kinase-1 (SphK1)/S1P pathway as a therapy target in prostate cancer. In the current study, we demonstrate that FTY720 can radiosensitize prostate cancer in cell and animal models.

FTY720 induced apoptosis in several hormone- and radioresistant prostate cancer cell lines. FTY720 treatment induced a sustained inhibition of SphK1 and a decrease in the intracellular S1P content. Enforced expression of SphK1 in prostate cancer cells rendered them resistant to FTY720. *In vitro* sub-lethal concentrations of FTY720 dramatically sensitized PC-3 cells to radiotherapy.

Mice orthotopically engrafted with fluorescent PC-3 cells were treated with FTY720 with or without γ-irradiation. FTY720 demonstrated a synergy with γ-irradiation (notably through SphK1 inhibition), significantly reducing tumor size, angiogenesis and formation of micrometastases as demonstrated by fluorescent *in vivo* imaging.

In conclusion, FTY720 can sensitize prostate cancer cells to radiotherapy both *in vitro* and *in vivo*, through inhibition of SphK1 and modification of intracellular levels of lipid second messengers.

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THE SK1/S1P PATHWAY AS A POTENTIAL DIAGNOSTIC/PROGNOSTIC MARKER IN PROSTATE CANCER

Maria Naymark, Lysann Sauer, Jonathan Waxman and Dmitry Pshezhetskiy

Department of Oncology, Imperial College London, London, UK

The sphingosine kinase-1/sphingosine-1-phosphate (SK1/S1P) pathway regulates several fundamental processes that are integral to cancer pathogenesis (cell proliferation, resistance to apoptosis, angiogenesis and the pro-inflammatory response). In the current study using a prostate cancer model, we hypothesize that elevation of SK1/S1P can serve as a potential diagnostic/prognostic marker.

In a preclinical study on 30 prostate cancer patients undergoing radical prostatectomy, we have demonstrated that SK1 is strongly up-regulated in human prostate tumours in comparison to non-tumour controls. SK1 elevation correlated with the Gleason score and the advanced stage of the disease. In several patients, prostate cancer cells obtained from known lymph node metastases had elevated activity of SK1 in contrast to the cells in the primary tumour providing a link between the SK1 activation and acquiring of the metastatic potential. Mice xenografted with human prostate cancer cells had increased levels of blood serum extracellular S1P in comparison with mice not bearing prostate tumours. Following docetaxel treatment, serum levels of S1P fell in parallel with the reduction of tumour volume.

In this study, for the first time we demonstrate that the SK1 activity and expression are correlated with the onset, progression and metastasis of human prostate cancer. Additionally, using an animal prostate cancer model we show that the levels of secreted S1P correspond to the tumour burden, which provides a rationale for proposing S1P as a novel tumour marker assessable in blood serum. Although not prostate specific, when combined with other methods, the SK1/S1P test may have a significant diagnostic and prognostic value.

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ESCAPE FROM FAILSAFE PROGRAMS BY TWIST ONCOPROTEINS AND EMT

Alain Puisieux

INSERM U590 Centre Léon Bérard, Université Claude Bernard Lyon 1, 69373 Lyon Cedex 08, France

A major obstacle to the expansion of abnormal cells with aberrant proliferative potential is the induction of innate defense mechanisms that initiate the cellular failsafe programs of senescence or apoptosis. The mechanisms by which pre-cancerous cells escape these protective barriers remain to be determined. Recently, we identified the Twist proteins as potential early drivers of tumorigenesis (1). Twist1 and Twist2 proteins are highly conserved basic helix-loop-helix (bHLH) transcription factors that have important regulatory functions during embryogenesis. Twist1 and/or Twist2 overexpression is a frequent event in multiple solid human tumors including many types of carcinomas as well as sarcomas, gliomas, neuroblastomas, and melanomas. Twist proteins inhibit both Rb- and p53-dependent pathways, thereby preventing apoptosis and oncogene-induced senescence (1, 2). Strikingly, in epithelial cells, the failsafe program escape facilitated by Twist1 or Twist2 coincides with complete epithelio-mesenchymal transition (EMT), a process associated with the acquisition of stem cell properties and invasive potential (1, 3). Collectively, these observations suggest that some metastatic capabilities of cancer cells can be acquired during malignant conversion as a side-effect of the inactivation of primary failsafe mechanisms.

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PULSE IL-2 WITH FAMOTIDINE AND CYCLOPHOSPHAMIDE HAS ACTIVITY IN PREVIOUSLY TREATED METASTATIC MELANOMA

Walter Quan Jr., Charles Knupp, Darla Liles, Francine Quan, Linda King and Paul Walker

Loma Linda University School of Medicine, Loma Linda, CA;
Leo Jenkins Cancer Center, East Carolina University School of Medicine, Greenville, NC, USA

Interleukin-2 (IL-2) is able to induce T-lymphocyte cytotoxicity against melanoma *in vitro* and *in vivo*. Famotidine may further enhance the activity of T-cells by allowing for increased Interleukin-2 internalization by the IL-2 receptor on lymphocytes. Previously, we reported the activity of IL-2 in combination with famotidine in stage IV melanoma (Quan, 2007). Cyclophosphamide may decrease the immunosuppressive effects of regulatory T-cells. We utilized daily short intravenous infusions (pulses) to treat 13 patients with metastatic melanoma. Patients received $21.6x10^6$ IU/m^2 pulse IL-2 intravenously over 15-30 minutes preceded by famotidine 20 mg *i.v.* daily for 5 days. Twelve patients received cyclophosphamide 350 mg/m^2 intravenously on day 1 (1 patient did not). Nine patients were treated at an oncology inpatient unit while, most recently, 4 have received therapy on an outpatient basis. Cycles were repeated every 3 weeks until disease progression. Patient characteristics: 7 males, median age-53 (range: 31-87) years, median ECOG performance status-1 (range: 0-1). Common metastatic sites: lymph nodes, 13; lungs, 9; subcutaneous, 4; liver, 3. Prior systemic therapy: IL-2, 10; interferon, 5; chemotherapy, 5; none, 2. Median number of cycles received (range) = 2 (1-7). Most common toxicities were fever, rigors, nausea/emesis, hypomagnesemia and hypophosphatemia. One complete and three partial responses were seen (31% response rate; 95% confidence interval: 17-60%). Responses occurred in lung, liver, lymph nodes, and subcutaneous sites. Median response duration = 3.4 months. Median survival = 9 months for the entire group. Seven patients remain alive with a median survival >10.5 months. Pulse Interleukin-2 with famotidine and cyclophosphamide has activity in previously treated patients with melanoma and may be given on an outpatient basis to selected individuals.

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CANINE MAMMARY TUMOURS AS A MODEL TO STUDY HUMAN BREAST CANCER: RECENT FINDINGS

Felisbina Luisa Queiroga

CECAV, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

Clinical and molecular similarities between canine mammary tumours (CMT) and human breast cancer have been described. Clinically, the similarities are very strong: spontaneous tumours, hormonal aetiology, age of onset and identical clinical course of the disease. The clinical characteristics that have an impact on the clinical outcome are also identical: tumour size, growth rate, lymph node invasiveness and clinical stage.

In both species, the search for prognostic factors to define those patients with higher risk of developing recurrence or death due to the tumour is essential. Nevertheless, nowadays as far as human medicine is concerned, the goal is to identify prognostic factors that could be used as therapeutic targets to support a better outcome in the clinical management of the neoplastic disease. Moreover, in this area, CMT seems to mimic human breast cancer. In fact, some of the recently established prognostic factors are molecular targets of new anticancer therapies acting as predictive factors that can identify patients which might benefit from specific anticancer drugs.

Clinical and molecular data that support CMT as a model to study human breast cancer are analysed in this review. Additionally it is shown that some recent molecular targets in CMT may be seen as indicators for similar research to be performed in the corresponding human disease.

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ACTIVATION/MATURATION OF CANINE CUTANEOUS HISTIOCYTOMA CELLS: A SWITCH TO ITS REGRESSION?

I. Pires[1], F.L. Queiroga[1], A. Alves[1], P. Rodrigues[1], F. Silva[1] and C. Lopes[2]

[1]CECAV, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real;
[2]Institute of Biomedical Sciences, Porto, Portugal

Canine cutaneous histiocytoma (CCH) is an epidermotropic Langerhans cell histiocytosis which occurs mostly in young dogs. The regression phenomena, to which it is associated, makes it an attractive system for analysis of Langerhans cell histiocytosis behaviour and could be regarded as a unique model to understand the pathogeny of the enigmatic disease of human Langerhans cell histiocytosis.

In order to contribute to the understanding of the CCH regression, 93 tumours were analysed. We evaluated the immunoexpression of MHC class II molecules and E-cadherin in tumoural cells. MHC class II were also studied by immunoelectron microscopy methods. Inflammatory infiltrate were also analysed (CD3, CD79 and MAC387).

In the study of the immunocytochemistry of MHC class II antigen, visualized by light microscopy and by electronic microscopy, two principal patterns of MHC class II antigens expression were observed: a focal juxtanuclear cytoplasmic reaction associated to endosomal and lysosomal compartments, or a rim-like staining in the cell periphery associated to membranes outlines. The first occurred, principally, in tumours with poor lymphoid inflammatory infiltrate and the second in tumours with abundant lymphoid inflammatory infiltrate, either by T-lymphocytes or B-lymphocytes. These findings seem to reflect different phases of MHC class II biosynthesis during activation/maturation of normal Langerhans cells.

The intensity of the immunoreaction to E-cadherin decreased with lymphoid infiltration. The decreased of E-caherin expression was also associated with a membranar expression of MHC II molecules.

This data suggests that pathological Langerhans cells in CCH appears in an immature state in early lesions and undergo a maturation process, characterized by an increase of membranar MHC class II molecules and a decrease in E-cadherin expression. In the regression lesions these cells had a mature phenotype and could interact them self with B and T-lymphocyte. CCH is a dynamic lesion that changes its phenotype and determines its own regression.

In dogs and humans, Langerhans cell histiocytosis has a distinct clinical behaviour. In dogs, regression is the natural course of the process. However, in humans, only a limited number of lesions undergo regression. The activation/maturation of pathological Langerhans cells could be one explanation for this fact.

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ENDOPLASMIC RETICULUM-RESIDENT HEAT-SHOCK PROTEIN GP96 AS REGULATOR OF NORMAL AND MALIGNANT GROWTH

Biserka Radosevic-Stasic, Hrvoje Jakovac, Damir Grebic and Ines Mrakovcic-Sutic

Department of Physiology and Immunology, Medical School, University of Rijeka, 51000 Rijeka, B. Branchetta 22, Croatia

Background: Various kinds of tissue disintegration lead the endoplasmic reticulum (ER) to activation of adaptive pathways known as the ER stress response, which is directed to correction of unfolded proteins, to the activation of proteasome-dependent

ER-associated degradation of the misfolded proteins and to activation of protein translation that modulate the polypeptide traffic into the ER. A crucial role in these events is played by gp96, which acts not only as a molecular chaperone, but also as an adjuvant, able to induce the specific immune response against some self and foreign antigens. *Materials and Methods:* To illustrate the multipotential functions of gp96 in this study, we investigated its participation in conditions of: 1) normal growth (liver regeneration after partial hepatectomy, pregnancy and fetal organogenesis), 2) autoimmunity (experimental allergic encephalomyelitis) and 3) pre-malignant growth (cervical intraepithelial neoplastic lesions). Tissue expression of gp96, detected by immunohistochemistry and RT-PCR in animal models was correlated with phenotype and cytotoxicity of hepatic and splenic mononuclear lymphatic cells against the NKT-sensitive (syngeneic thymocytes) and NK-sensitive targets (YAC-1 cells), while in CIN lesions (gradus I-III) it was correlated with the intensity of the neoplastic process. *Results:* Tissue expression of gp96 was highly up-regulated in fetal and adult rapidly proliferating tissue (regenerating liver and in several fetal organs), at the fetoplacental barrier, in injured tissues after autoimmune attack (on astrocytes, microglia and neurons in the brain and spinal cord during EAE), as well as in dysplastic areas of cervix (on hyperplastic epithelial cells, vascular epithelia, and glandular epithelium). Kinetic studies made in the model of liver regeneration also showed that gp96-overexpression in the liver and thymus was followed by maturation of dendritic cells, accumulation of $CD3^{intermediate}/NK1.1^{+}/CD69^{+}$cells in the liver and $CD4^{+}CD25^{+}Fox3^{+}$ cells in the liver and thymus, as well as by augmentation of NKT- and NK-mediated cytotoxicity in the liver and in the spleen. *Conclusion:* The data imply that during the disturbance of morphostasis and structural integrity of the cell gp96 may protect the cells against the proteotoxic damage and serve as a natural adjuvant for chaperoning of antigenic self or oncogenic peptides into the immune surveillance pathways, contributing during the normal growth to survival of cells and reestablishment of self tolerance, and in pathological growth to both development of cancer and initiation of antitumour immune response.

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LYMPHANGIOGENESIS AS A POTENTIAL TARGET FOR TUMOR THERAPY

Marius Raica and Anca Maria Cimpean

"Victor Babes" University of Medicine and Pharmacy, Department of Histology and Cytology, Romania

Lymphangiogenesis is the process of lymphatic vessel (LV) formation, but despite the interest on this topic in physiological and pathological conditions, the mechanisms are still to be elucidated. Renewed interest in the lymphatic system began less than 10 years ago and was based on the introduction of some specific markers of lymphatic endothelial cells (LECs). Various human tumors preferentially metastasize by a lymphatic route and lymphovascular invasion is known for years as an element that accurately predicts lymph node metastasis. On the other hand, the intimate mechanism/s by which tumor cells initially enter LVs is largely unknown. In this context, there are several questions (*Q*) to be answered regarding tumor lymphangiogenesis.

Q1: How specific are LEC markers? The most frequently used LEC markers are VEGFR3, LYVE-1, Prox-1, and podoplanin. It was shown that in postnatal life, these markers are expressed by LECs and not by blood vessel ECs. They have no absolute specificity, as their expression was reported in some normal and pathological cells/tissues.

Q2: Can LVs be found in tumors? The best results were obtained with D2-40 that recognizes an epitope of podoplanin. Peritumoral LVs were found in a large variety of tumors and intratumoral LVs were reported in SCC, melanoma, urothelial and gastric carcinoma. In patients with breast cancer, specific identification of LVs increased detection of lymphovascular invasion from 11 to 26%.

Q3: Du tumor associated-LVs preexist or are they newly formed? Do they have proliferative potential? Proliferating LECs were demonstrated by double immunostaining (MIB-1/Ki67) in SCC, pancreatic endocrine carcinoma, and malignant melanoma.

Q4: Does lymphatic microvascular density (LMVD) have a real prognostic impact? Present data are controversial. LMVD correlates with the risk for metastasis in SCC, melanoma, endometrial, uterine cervix, pancreatic and gastric carcinoma. No correlation was found in cases with hepatocellular and ductal pancreatic carcinoma, and divergent results were obtained for breast cancer.

Q5: What is the status of the main lymphangiogenic factors (VEGF-C/D) and of their specific receptor (VEGFR3)? All data strongly support the major role of the VEGF-C – VEGFR3 axis in induction of lymphangiogenesis. VEGF-C may be secreted by tumor cells themselves and/or by activated macrophages. VEGF-C overexpression in tumors induces LV hyperplasia.

Q6: What is the significance of LECs specific markers expression by tumor cells? The best example is represented by podoplanin expression in mesothelioma, angiosarcoma, Kaposi sarcoma, seminoma, diffuse gastric carcinoma, mastocytoma, and some tumors of the brain. Podoplanin expression by tumor cells is usually associated with invasion and progression and in some cases is useful in the differential diagnosis. Could it be a potential therapeutic target? At present, the answer is no, because it is also expressed by many normal cells.

Q7: Can lymphangiogenesis be inhibited? Preliminary results in experimental models using anti-VEGF-C, anti-VEGFR3, or rapamycin showed the reduction in the rate of lymph node metastasis and a decrease in LMVD. Therefore, attractive targets for cancer therapy could be both LVs and some tumor cells.

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COMPARATIVE THERAPEUTIC AND ADJUVANT EFFECTS OF CURCUMIN AND TURMERIC FORCE™ AGAINST HUMAN CANCER

C. Ramachandran, A. Resek, E. Escolan and S.J. Melnick

Department of Pathology, Miami Children's Hospital, 3100 SW 62nd Avenue, Miami, FL 33155, USA

Curcumin, the yellow colored chemotherapeutic agent from the Indian spice turmeric (*Curcuma longa*) has antitumor, anti-inflammatory, immunostimulative and antioxidant effects. Of the 214 apoptosis-associated genes studied in cancer cells, we found that 104 genes were altered by curcumin. Despite its anticancer effects, one of the major problems with the therapeutic use of curcumin is its poor biological availability and limited efficacy. Our studies have also shown that curcumin and doxorubicin combination treatments have synergistic cytotoxicity, with combination index values less than 1 in human leukemia cell lines. These two compounds in combination induced increased G_2/M arrest and apoptosis in tumor cells. While DOX treatment up-regulated NF-κB in both CEM and CEM/VLB cell lines, curcumin inhibited the NF-κB activity.

Turmeric Force™ (TF) is the supercritical CO_2 and hydroethanolic extract of turmeric rhizomes that is marketed as a nutraceutical by New Chapter Inc., VT, USA. It contains 45% turmerones, 11% curcuminoids and other compounds that may represent the full potency of turmeric, unlike curcumin. TF is equally cytotoxic in tumor cell lines with IC_{50} values ranging from 6.7-12.6 μg/ml. MDR cells do not have any resistance towards TF, whereas normal lymphocytes are generally protected against this agent. TF inhibits NF-κB activity and IL-8 expression leading to induction of apoptosis in tumor cell lines. TF showed better cytotoxicity than curcumin in two pancreatic carcinoma cell lines (Bx-PC3 and Panc-1), with mean IC_{50} values of 1.00 and 1.22 μg/ml, respectively. Gemcitabine, which is currently used as standard chemotherapeutic agent against pancreatic cancer, has an IC_{50} value of 0.03 ug/ml, although the higher concentration (50 μg/ml) was unable to induce not more than 60% cell death. When the cytotoxic data were analyzed by CalcuSyn software, gemcitabine and TF combination showed strong synergism, with combination index (CI) values of 0.216 and 0.236 in BxPC3 and Panc-1 cell lines, respectively. Flow cytometric analysis of DNA content using propidium iodide staining showed that TF induced G_2/M block and a sub G_0/G_1 population in a dose-dependent manner. Data on cell cycle arrest and annexin–V staining confirmed the involvement of apoptosis on TF cytotoxicity in tumor cells. Both gemcitabine and TF down-regulated NF-κB activity and the combination treatment showed a higher degree of inhibition than either agent alone, although the NF-κB mRNA level remained the same in all treatment groups. Our *in vitro* studies indicate that TF can be used as a single agent or an adjuvant with gemcitabine for the treatment of pancreatic carcinomas. Since TF contains other molecules, especially turmerones, and represents the full potency of raw turmeric, TF may be better than curcumin and more biologically available.

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PRE-CLINICAL STUDIES ON A NOVEL IMMUNE-STIMULATING POLYSACCHARIDE FROM *TINOSPORA CORDIFOLIA*

C. Ramachandran, P.K. Raveendran Nair, E. Escolan and S.J. Melnick

Department of Pathology, Miami Children's Hospital, Miami, FL 33186, USA

Polysaccharides are known immune stimulants, of which beta-glucans have received considerable attention. We have isolated and characterized an α-D-glucan (RR1) that is comprised of 1-4 linked back bone and 1-6 linked branches with a molecular mass of 550 kDa from the medicinal plant *Tinospora cordifolia*. This novel polysaccharide is non-cytotoxic and non-proliferating to normal lymphocytes as well as tumor cell lines, even up to 1 mg/ml. RR1 activates subsets of lymphocytes such as natural killer (NK) cells (331%), T- (102%) and B-cells (30%) at 100 μg/ml. Immune activation by RR1 in normal lymphocytes elicited the synthesis of interleukin (IL)-1β, IL-6, IL-12p70, IL-12p40, IL-18, IFN-γ, TNF-α and MCP-1, while it did not induce the production of IL-2, IL-4, IL-10, INF-α or TNF-β. The cytokine profile clearly demonstrates the Th1 pathway of T-helper cell differentiation essential for cell-mediated immunity with a self-regulatory mechanism for the control of its overproduction. RR1 stimulation did not produce any oxidative stress or inducible nitric oxide synthase (iNOS) in the lymphocytes or any significant increase in nitric oxide production. The water solubility, high molecular mass, activation of lymphocytes especially NK cells, complement activation, Th1 pathway-associated cytokine profile, together with a low level of nitric oxide synthesis and absence of oxidative stress confer immunoprotective potential on this novel α-D-glucan. RR1 inhibits the phagocytosis of unopsonized zymosan A bioparticles in a dose-dependent manner in macrophages. Incubation of macrophages with anti-CD11b mAb followed by RR1 failed to show any inhibitory

effect on RR-1-induced TNF-α synthesis, confirming that complement factor 3 (CR3) is not involved in the opsonic binding and internalization of RR1 in macrophages. RR1 activated NF-κB in a time- and dose-dependent manner and this modulation is associated with degradation of I-κB-α thus facilitating the translocation of NF-κB into the nucleus. RR1-induced NF-κB activity peaks at 8 h of RR1 stimulation while I-κB alpha degradation occurred within 1 h of stimulation. RR1-induced NF-κB activation occurred through TLR6 signaling as evidenced by the synthesis of IL-8 in TLR6-transfected HEK293 cells. These results show that the novel (1,4) α-D-glucan from *T. cordifolia* activates the immune system through the activation of macrophages that occurs through TLR6 signaling, NF-κB translocation and cytokine production. Our preclinical studies indicate the potential use of RR1 as an adjuvant against human malignancies which may work through modulation of native immunity.

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MDA-7/IL-24: A NOVEL GENE DRUG FOR HUMAN CANCER THERAPY

Rajagopal Ramesh[1], Manish Shanker[1], Tomohisa Yokoyama[1], Cynthia D. Branch[1], Mingzhong Zheng[2], Ailing W. Scott[1], Jiankin Jin[1], Dora Bocange[1,2] and Sunil Chada[2]

[1]Department of Thoracic and Cardiovascular Surgery, M.D. Anderson Cancer Center, Houston, Texas, 77030; [2]Introgen Research Institute, Houston, Texas 77030, USA

The melanoma differentiation-associated gene-7 (mda-7), also known as interleukin-24, is a unique tumor-suppressor/cytokine that belongs to the interleukin-10 (IL-10) family. MDA-7/IL-24 encodes a protein of 206 amino acids (a.a) in length and has limited homology (19%) with IL-10 protein, and appears to function antagonistically to IL-10. MDA-7/IL-24 protein, unlike IL-10 or other cytokines, undergoes extensive post-translational modifications and exhibits phosphorylation, glycosylation and ubiquitination. The modifications are frequently reported for tumor suppressor proteins and regulate function and potency. Glycosylated MDA-7/IL-24 is secreted and binds to two heterodimeric receptors, termed type 1 and type 2 IL-20 receptors (IL-20R1/IL-20R2 or IL-22R1/IL-20R2). Detection of endogenous MDA-7/IL-24 protein has not been reported to date in tumor cell lines (>60 lines evaluated). However, immune cells upon stimulation with PHA, LPS or IL-2 family cytokines potently induce expression of MDA-7/IL-24 protein or mRNA in T-cells and monocytes.

Studies have shown that mRNA but not the protein product of *mda-7* is detectable in human lung cancer and melanoma cell lines. Additionally, an inverse correlation between MDA-7 protein expression and cancer progression and patient survival has been reported in both lung cancer and melanoma, suggesting MDA-7/IL-24 is a novel tumor suppressor protein. Further evidence was provided in preclinical studies when exogenous expression of MDA-7/IL-24 protein using adenovirus and nanoparticles selectively killed human cancer cells both *in vitro* and *in vivo*, with little to no effect on normal cells. Additionally, MDA-7/IL-24, when combined with chemotherapy, radiation therapy or biological therapies, demonstrated enhanced killing of tumor cells. The molecular mechanism of tumor cell killing is cell-type dependent and culminates in apoptotic cell death. Furthermore, MDA-7/IL-24 showed antiangiogenic activity both *in vitro* and *in vivo* which was enhanced when combined with other antiangiogenic agents, such as bevacizumab (avastin). All of these features classify MDA-7/IL-24 as a tumor suppressor protein-cytokine and a promising cancer drug.

Preclinical studies in our laboratory have focused on investigating the role of secreted MDA-7/IL-24 protein on anticancer activity, molecular mechanisms and the contribution of receptor-mediated cell death. Analysis of receptor expression by RT-PCR and Western blot in human cancer cells identified receptor-negative (H1299, A549) and receptor-positive (*e.g.* MeWo, A375, 2008, AsPC3) cell lines. Treatment of tumor cells with MDA-7/IL-24 protein showed dose-dependent and time-dependent killing of receptor-positive, but not receptor-negative cells. Furthermore, blocking studies using neutralizing antibodies to the receptors or MDA-7/IL-24 protein resulted in abrogation of the killing effect. Molecular analysis showed transient activation of STAT-3 upon ligand binding to its receptors. These results showed MDA-7/IL-24 protein kills tumor cells in a receptor-dependent manner. However, the requirement of type 1 or type 2 IL-20 receptors varied and was observed to be cell-type dependent. Treatment of receptor-positive normal cells with MDA-7/IL-24 protein showed no cell killing, suggesting intracellular signaling following ligand-receptor binding is different in normal and tumor cells. Similarly, treatment of receptor-positive human microvascular endothelial cells (HMVECs) and human umbilical vein endothelial cells (HUVECs) with the protein *in vitro* inhibited differentiation, tube-formation, cell migration and invasion. The antiangiogenic activity was mediated *via* the IL-22R1 subunit of type 2 IL-20R. *In vivo*, MDA-7/IL-24 protein inhibited tumor angiogenesis, resulting in delay of tumor growth. Thus, secreted MDA-7/IL-24 protein is selective for receptor-positive cells and kills only tumor cells while sparing normal cells. The sensitivity of receptor-positive tumor cells and resistance of receptor-positive-normal cells to MDA-7/IL-24 appears to be regulated by differences in the intracellular signaling following ligand–receptor interaction and warrants further investigation.

A Phase I clinical trial testing an adenovirus-based mda-7 (Ad-mda7; INGN241) gene drug for cancer therapy showed patients tolerated the drug well with no significant toxicity,

with clinical responses observed in few patients. In conclusion, MDA-7/IL-24 with potent tumor killing activities is a novel cancer drug that warrants further clinical testing.

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VIRUS-LIKE PARTICLES (VLPS) CARRYING TUMOUR-ASSOCIATED ANTIGENS AS TUMOUR-SPECIFIC VACCINES EXEMPLIFIED FOR HER2-EXPRESSING TUMOURS

Torbjörn Ramqvist

Department of Oncology-Pathology, Karolinska Institutet, Cancer Centrum Karolinska, SE-171 76 Stockholm, Sweden

Vaccines based on virus-like particles (VLPs) have recently come into special focus by the use of VLP-based vaccines against human papilloma viruses (HPV). However, this application of VLPs is just one of many possible uses for VLPs both in the field of tumour prevention and as vaccines against different viruses. Another type of application is to utilize VLPs as antigen carriers for different tumour antigens, both of viral and non-viral origin for the use as tumour vaccines in preventive or therapeutic settings. We have constructed VLPs based on different types of murine polyoma viruses carrying the extracellular and transmembrane domain of human Her2/*neu* fused to the polyoma capsid protein VP2. One single injection of these Her2PyVLPs efficiently immunized against a Her2-carrying tumor in a transplantable model and against the development of mammary cancer in mice transgenic for mutated rat *neu* gene. We have further improved the immune response by loading Her2PyVLPs into dendritic cells (DCs) and by inoculating Her2PyVLPs together with CpG. Inoculation of Her2 VLPs together with CpG strengthens the *in vivo* response and confers protection against Her2 carrying tumours also in a therapeutic setting.

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A NEW ANTI-TUMOUR VACCINATION STRATEGY: ADENOVIRAL VECTOR ENCODING INVARIANT CHAIN LINKED TO THE TUMOUR ASSOCIATED ANTIGEN

Maria Rathmann Sørensen, Peter Johannes Holst, Jan Pravsgaard Christensen and Allan Randrup Thomsen

University of Copenhagen, Institute of International health, Immunology, and Microbiology, The Panum Institute, Build. 22.5, Blegdamsvej 3c, 2200 Copenhagen N, Denmark

Antigen-specific immunotherapy is an attractive strategy for cancer control. In the context of antiviral vaccines, adenoviral constructs have emerged as the most favourable means for immunization. For this reason, we have selected an innovative approach that combines the use of this vector system with another successful approach, namely linkage of the relevant antigen to the invariant chain (Ii). To test this vaccination strategy we used a mouse model system, in which a an epitope of the glycoprotein (GP33-41) of a virus (lymphocytic choriomeningitis virus (LCMV)), works as a tumour associated antigen (TAA). Mice (C57BL/6) vaccinated prophylactic with adenovector expressing GP linked to Ii (Ad-Ii-GP) were completely protected against growth of B16.F10 melanoma cells expressing GP33-41. Furthermore, a single therapeutic vaccination 5 days after tumour challenge delayed tumour growth by approximately 14 days compared to irrelevant vaccination. This tumour control was as good as a prophylactic or therapeutic "vaccination" with infectious virus. Therapeutic vaccination with the linked construct was moreover significantly better than vaccination with an adenovector expressing GP only (Ad-GP). The improved therapeutic vaccination efficiency of the linked vaccine appeared to be due to an increased TAA specific CD8 T cell response. Furthermore, vaccination experiments using mice deficient in expression of IFNγ, perforin, or both, indicated that the improved vaccination efficiency is dependent on IFNγ, which reduces cell cycle entry of the melanoma cells. In contrast, perforin was redundant in otherwise immunocompetent mice, but not in IFNγ deficient mice.

Tumour regression was followed by regained tumour growth, and this correlated with a contraction of the tumour specific CD8 T cells. To delay this contraction, and thus to further improve the therapeutic efficiency of the vaccine, we combined the vaccination with agonistic anti-CD40 antibodies to provide additional DC activation, and with anti-CTLA-4 antibodies to block CTLA-4 interaction with ligands on the APCs. Together with an additional Ad-Ii-GP vaccine booster, these antibody treatments improved the vaccination efficiency significantly, and about one third of the vaccinated mice became long-term non-progressors. From this we conclude that combination of a strong CD8 T cell inducing anti-tumour vaccine and antibody therapy inhibiting key immunosuppressive circuits might be a way to improve current protocols for immunotherapy of cancer.

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MICRORNA MIR-16 LINKED TO THE DEVELOPMENT AND PROGRESSION OF B-CELL MALIGNANCIES IN THE NZB MURINE MODEL OF CHRONIC LYMPHOCYTIC LEUKEMIA

Erica Salerno[1], Brian Scaglione[1], Frederick Coffman[1], Helen Fernandes[1], Gerald Marti[2] and Elizabeth Raveche[1]

[1]University of Medicine and Dentistry of New Jersey – Newark, NJ 07103;
[2]NIH, Bethesda, MD 20812, USA

The NZB mouse serves as a *de novo* model for CLL and *in vivo* imaging has been performed by CT and MRI to observe

splenomegaly and lymphadenopathy. Alterations in the 13q14 genomic region containing microRNAs miR-15a and miR-16-1 have been found in human CLL, resulting in the deletion or down-regulation of these miRNAs in 60% of patients. We found the development of CLL in NZB backcrosses was linked to a point mutation in the 3' flanking region of mir-16-1, similar to the one described in human CLL. In *ex vivo* NZB lymphoid tissue, as well as in an NZB-derived *in vitro* malignant B cell-line (LNC), the levels of mature miR-16 are decreased by half relative to levels in non-NZB control strain spleens or lymphoid cell lines. Lower expression of miR-16 results in increased expression of miR-16 target genes, such as *CCND1* (cyclin D1), contributing to uncontrolled tumor growth. When NZB malignant B-cells are separated by cell cycle phase (by elutriation), and then transfected with exogenous miR-16, lower proliferation is observed and an accumulation in G0/G1 phase with the most sensitive phase in the S/G2 fraction. In addition, when miR-16 was added back to LNC, the cyclin D1 levels decreased, returning to the normal range. Spleens and lymph nodes from NZB and control mice were sorted by FACS into conventional B220 bright B-cells or malignant B220 null B-1 cells. Both sources of NZB B-cells had lower miR-16 expression relative to C57Bl/6 purified B cells. Interestingly, among NZB B cells, the malignant B-cells had higher expression of miR-16 than did the non-malignant B-cells (B220 bright). In addition, spleens, peritoneal cells and lymph nodes from old NZB mice exhibited a dramatic increase in the 'side population' present relative to young NZB and control mice, suggesting an increase in cancer stem cells as NZB mice age. Our data further support that the miRNA, miR-16, has an important role in the development and progression of B-cell malignancies. The presence of the increased 'side population', reduced miR-16 levels, and the miR-16-1 3' flanking region mutation in NZB present clues to CLL development.

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AGE-ASSOCIATED INCREASE IN CANCER STEM CELLS DETECTED AS THE SIDE POPULATION IN THE NZB MODEL OF CLL

Elizabeth Raveche[1], Sivasubramanian Baskar[2], Erica Salerno[1], Brian Scaglione[1], Christoph Rader[2], Steven Bauer, Rameet Hundle[1] and Gerald Marti[2]

[1]University of Medicine and Dentistry of New Jersey – Newark, NJ 07103;
[2]NIH, Bethesda, MD 20812, USA

As NZB mice age they develop a malignant clonal expansion of B-cells and serve as a *de novo* model of B-cell chronic lymphocytic leukemia (CLL). Analysis of the spleens from old NZB mice demonstrated the presence of a unique population of cells that are able to efflux the DNA-binding dye, Hoechst 33342, and are referred to as the side population (SP). The SP was lost in the presence of verapamil, which prevents efflux of Hoechst and defines the SP. In addition, most of these SP cells expressed cell surface markers of stem cell progenitors such as c-Kit and/or Sca-1. The percentage and absolute number of SP cells in spleen, lymph node, peritoneal cavity, blood and bone marrow of old (12-month) NZB was increased when compared to young (1- to 3-month) NZB. By contrast, old DBA/2 and C57Bl/6 control mice, which do not develop leukemia, had low levels of SP cells in all the organs tested. The increase in SP cells in old NZB mice correlate with the development of CLL and the SP cells may be cancer stem cells which are resistant to apoptosis.

To further study the SP, the spleen cells from old NZB mice were sorted into non-SP and SP cells and subjected to microRNA(miR) analysis by qRT-PCR. Previously, we had shown the development of CLL in NZB backcrosses was linked to a point mutation in the 3' flanking region of mir-16-1. A similar mutation in the 13q14 region of human CLL containing the mir-16-1 has been described by others. In the present studies, we found that the sorted NZB SP cells had lower expression of miR-16 when compared to the non-SP cells. Decreased miR-16 in the NZB SP population resulted in increased expression of miR-16 target genes in the NZB SP cells, many of which could contribute to uncontrolled tumor growth and failure to undergo apoptosis. Our data further supports that miR-16 has an important role in the development and progression of B-cell malignancies. The presence of the increased 'side population' in old NZB tissue sources suggest that these cells may be a target for the elucidation of new strategies to control CLL growth.

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HORMONAL CHANGES AS POTENTIAL BIOMARKERS OF PROGNOSIS IN HEAD AND NECK SQUAMOUS CELL CANCER PATIENTS

É. Remenár, B. Vincze, I. Számel, B. Budai, A. Ladányi, J. Timár and M. Kásler

National Institute of Oncology, Budapest, Hungary

Background: Head and neck squamous cell cancer (SCCHN) is characterized by rapid progression and poor prognosis. The most reliable prognostic factors are tumor (T) and node (N) stage. There is some evidence that circulating hormone levels might predict prognosis in SCCHN. The aim of this study was to assess hormone levels in 289 male patients and evaluate them in association with the clinical parameters. *Methods:* Age, primary tumor site, tumor stage, histologic grade, and serum levels of estradiol (E2), progesterone (PROG), testosterone (TE), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHAS), steroid hormone

binding globulin (SHBG), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PROL) of 289 patients operated for cancer of the oral cavity, oro-, and hypopharynx and the larynx in one cancer center were recorded. The median follow-up was 37 months (19-71). *Results:* Age <46 years *vs*. older (p=0.0055), stage I-II vs. III (p=0.014), and stage III *vs*. IV (p=0.0004), N0 *vs*. N+ stage (p<0.0001) and Gr.I-II *vs*. Gr.III histology grade (p=0.03) were of prognostic importance. Of the hormones studied, lower than normal levels of TE (p=0.02), TE/SHBG (p=0.0031) and DHAS (p=0.04), as well as high levels of FSH (p=0.022), LH (p=0.0083) and PROL (p=0.0043) predicted poor prognosis. In multivariate analysis of all cases, T stage, N stage and grade proved to be independent prognostic factors, PROL level had borderline significance (p=0.07). In the N0 stage subgroup PROL (p=0.0013), and in the N+ stage subgroup histology grade (p=0.0004) and TE (p=0.0053) emerged as independent prognostic factors by multivariate analysis. *Conclusion:* Our results suggest that abnormal levels of some circulating hormones in head and neck cancer predicts worse survival, therefore hormonal imbalance cannot be excluded to have a role in the course and/or development of HNSCC.

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A NEW CANCER RESISTANT MOUSE MODEL (SR/CR)

Klaus Rieneck

University Hospital of Copenhagen, Denmark

The SR/CR mouse model of cancer resistance was fortuitously discovered by Cui and co-workers (2003). This mouse strain has been shown to be resistant to various tumour cell lines, which cause cancer in other mouse strains into which they have been administered. The mouse model constitutes an opportunity to attain insight into the interplay between cancer and the innate immune system. Through a more detailed immunological and genetic examination of this mouse model, novel anti-tumour immunological mechanisms may be unravelled.

What makes the SR/CR mouse unique in cancer research is its ability to specifically target and kill a number of different injected cancer cells, and thereby prevent the cancer development without harming normal host cells. The tumour resistance of the SR/CR mouse has been shown to be mediated by three innate immune cell populations, *i.e*. neutrophils, NK cells and macrophages. The SR/CR phenotype is transferable to wild-type mice by adoptive transfer and established tumours in wild-type mice will regress following transfer of these three cell types from SR/CR mice.

Furthermore the lifespan of the SR/CR mouse is normal without any signs of autoimmunity. The genetic basis of this phenotype is unknown but the phenotype is heritable as a mendelian trait. It is intriguing that older SR/CR mice, which are injected with cancer cells for the first time, will develop cancer and will regress completely after two-three weeks.

In summary the phenotype of the SR/CR mouse points to the importance of innate cellular immunity as a mediator of one or more significant anti-cancer mechanisms.

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BREAST CANCER AND CIRCULATING TUMOR CELLS

M. Tartary[1] and M. Rigaud[2]

[1]Laboratoire Astralab, Limoges;
[2]Faculté de Médecine de Limoges, France

Death of most patients is caused by metastatic spread of cancer cells from the primary tumor to distant organ. Some data indicated that approximatively 10^6 tumor cells per gram of tumor tissue per day shed into the blood stream (1). Tumor cell shedding is an early event in tumorogenesis (2).

A great number of cells move to the circulation but only a minority of them can form metastasis. One of 40 cells give rise to micrometastases and 0.01% proliferate into. Macrometastasis (3). In the blood stream a large fraction of circulating tumor cells (CTCs) die quickly, anoïkis being one of the major factors. To move to the circulation, tumor cells undergo the phenotype switch, "Epithelial to mesenchymal transition" (EMT) and then acquire mobile propensities (4). These CTCs are out of cell cycle and do not proliferate. Unrestrained CTCs' proliferation give rise to metastasis *via* the phenotypical reversion, "mesenchymal epithelial transition" and angiogenesis.

Although metastasis formation from CTCs is a highly inefficient process, as breast tumor size increases, their number in the blood increases and a fraction of them can evolve to a full-fledged metastasis (5). From 25,000 CTCs in the whole blood, 625 are candidate to give rise to a micrometastasis and at least 2 of them will be the origin of a metastasis. This could explain that the presence of CTCs in primary and metastatic breast cancer is associated with poor prognosis (6).

CTCs can be considered as new markers in oncology, but before introduction of their detection into clinical use, much work remains to be done. A major requirement is the standardization of detection systems (the different methods will be discussed) and the establishment of an agreement on threshold values. Another crucial step is the definition of optimal multi-markers assays, as no single ideal marker exists for CTCs detection. The choice of markers should take into consideration the cancer stem cell markers. In the near future, the establishment of the clinical usefulness of CTCs detection in oncology will be a challenge.

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MOLECULAR IMAGING, RADIATION THERAPY, AND THE MARRIAGE OF CADMUS AND HARMONY

Arturo Chiti[1], Marta Scorsetti[2], Marcello Rodari[1], Mario Bignardi[2], Pierina Navarria[2] and Gianluigi Ciocia[1]

[1]Nuclear Medicine Division and [2]Radiation Oncology Division, Istituto Clinico Humanitas, Milano, Italy

Positron Emission Tomography (PET) has been demonstrated to be of great value in the management of cancer patients. In recent years its use has been extended to the optimization radiation therapy treatment planning, with the aim of improving patient outcome. The radiopharmaceutical which is most often employed is the glucose analogue 18F-fluoro-deoxy-glucose (FDG). Several studies reported the usefulness of FDG-PET for RT target volume determination, particularly in non-small cell lung cancer (NSCLC). The use of FDG PET demonstrated to change the RT planning also in head and neck cancers, lymphoma and esophageal cancer. These applications are possible thanks to the use of integrated PET/CT images, acquired according to radiotherapy treatment position. Besides FDG other radiopharmaceutical can be used, like 11C-choline for prostate cancer and 11C-methionine for brain tumors. Some issues are of particular importance in order to have an accurate treatment planning. In particular, the optimization of images within the RT planning system and the rules implemented for contouring tumor volumes are the most critical issues.

The use of FDG PET in RT planning for some different types of cancer is now implemented in many centers in Europe and US, using different protocols for imaging and contouring. The advent of PET-CT hybrid imaging not only revolutionized the medical imaging, but also gave rise to a new and accurate method to increase accuracy of RT planning system. Although available data demonstrated the use of PET-CT in RT planning is able to modify the CT based RT planning in a high percentage of cases, no data are yet available to demonstrate that this change has an impact on patients outcome. In Humanitas we are currently using PET-CT as an aid to RT planning in NSCLC and brain tumors with promising results. The presentation will show the general needs for an effective use of PET in radiation planning and our experience in the field.

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ALTERED PROPERTIES OF PHOSPHORYLATED OR NITROSYLATED THYMIDYLATE SYNTHASE

Tomasz Fraczyk[1], Tomasz Ruman[2], Dagmara Rut[2], Elzbieta Dabrowska-Mas[2], Joanna Ciesla[1], Zbigniew Zielinski[1], Katarzyna Sieczka[1], Jacek Sikora[3], Elzbieta Walajtys-Rode[2], David Shugar[3] and Wojciech Rode[1,2]

[1]Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa;
[2]Rzeszow University of Technology, Faculty of Chemistry, Rzeszow;
[3]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, Poland

Thymidylate synthase (TS; EC 2.1.1.45), a major target in cancer chemotherapy, catalyzes the $N^{5,10}$-methylene-tetrahydrofolate (meTHF)-assisted C(5)-methylation of 2'-deoxyuridine-5'-monophosphate (dUMP), leading to formation of 2'-deoxythymidine-5'-monophosphate.

Possible phosphorylation of TS in cultured rat cells, previously reported (Samsonoff *et al*: J Biol Chem *272*: 13281-13285, 1997), prompted us to examine this in more detail. TS preparations from various sources, all highly purified in the presence of phosphatase inhibitors, included L1210 parental and FdUrd-resistant forms, as well as mouse, rat, human and *Trichinella spiralis* recombinant enzymes. They were analyzed, following SDS-PAGE, with the Pro-Q® Diamond Phosphoprotein Gel Stain, and all were found to include a low proportion of phosphorylated forms. However, MS analysis of the SDS-PAGE bands did not reveal any phosphorylated amino acid residues. By contrast, MS analysis of IEF fractions of TS preparations from parental and FdUrd-resistant mouse leukemia L1210 cells, whose differing sensitivity to inactivation by FdUMP and its analogues was previously found not due to mutations (Ciesla *et al*: Acta Biochim Pol *53*: 189-198, 2006), demonstrated phosphorylation of Ser10 and Ser16 in the resistant, but not the parental, enzyme.

Each of the four recombinant TS preparations, expressed in bacterial cells, was separated into phosphorylated and non-phosphorylated fractions, using metal oxide/hydroxide affinity chromatography on $Al(OH)_3$ beads, yielding phosphorylated fractions corresponding to ≈1% of the total. Each phosphorylated form exhibited a 3- to 4-fold lower V_{max}^{app}, but unaltered K_m^{app} with either substrate or cofactor relative to the non-phosphorylated form, and ability to repress translation (catalyzed by a rabbit reticulocyte preparation) of its own (as well as luciferase) mRNA. Surprisingly, MS analyses did not reveal the presence of phosphorylated amino acid residues in any of the fractions investigated. In striking contrast, ^{31}P NMR spectroscopy clearly demonstrated the presence of phosphorylated residues in the phosphorylated enzyme fractions, and their absence in non-phosphorylated. Further analyses of the ^{31}P NMR spectra (including their time-dependent changes following acidification), and comparison with those of synthetic phosphoramide derivatives of basic amino acids (Lys, Arg and His), and commercially available phospho-serine, phospho-threonine and phospho-tyrosine, revealed the presence of phosphorus in a phosphoramide (acid-labile) bond, pointing to modification of histidine residue(s).

Biological nitration of protein tyrosine residues, which may lead to modulation of protein function, is associated with many diseases, including cancer, and is correlated with intensified NO biosynthesis. Human, mouse and *Caenorhabditis elegans* recombinant TS preparations, each incubated *in vitro* in the presence of $NaHCO_3$, $NaNO_2$ and H_2O_2, underwent nitration of tyrosine residues, resulting in a distinctly diminished rate of the enzyme-catalyzed reaction. The V_{max}^{app} value was 2-fold lower when 1 tyrosine residue per monomer was nirated (with human or *C. elegans* TS) or 2 tyrosine residues per monomer (with mouse TS). No distinct effect on enzyme interactions with substrate (dUMP), cofactor (meTHF) or inhibitor (5-fluoro-dUMP) was apparent.

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566B

DIHYDROFOLATE REDUCTASE EXPRESSION IN METHOTREXATE-TREATED HUMAN ADENOCARCINOMA CELLS

Magdalena Dąbrowska and Wojciech Rode

Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa, Poland

Dihydrofolate reductase (DHFR) plays an important role in *de novo* nucleotide biosynthesis, and thus DNA replication, its expression growing at the entrance to the S phase of the cell cycle. Inhibition of DHFR results in cessation of thymidylate and purine synthesis, ultimately evoking a cytostatic effect (Schornagel and McVie: Cancer Treatment Rev *10*: 53-75, 1983). The classic antifolate methotrexate (MTX), a strong DHFR inhibitor, has been used in cancer chemotherapy for nearly 60 years, its chemotherapeutic effectiveness being hindered by resistance to the drug underlain by various mechanisms. DHFR translational autoregulation is assumed to constitute one of the main modalities of MTX resistance (Schmitz *et al*: Cancer Met Rev *20*: 33-41, 2001). Treatment of human adenocarcinoma C85 cells with 1 μM MTX was previously demonstrated to cause growth arrest in G1 phase of the cell cycle (Dąbrowska *et al*: Eur J Pharmacol *555*: 93-99, 2007). The present study was aimed at regulation of DHFR expression in the context of MTX treatment outcome.

Patterns of DHFR expression, determined by real-time PCR and Western blot techniques, and specific activity, were followed in C85 cells treated with 1 μM MTX for 48 h. The influence of MTX on putative DHFR translation autoregulation was monitored using a reporter system where 89-nt long DHFR region (designated L-region), identified elsewhere as sufficient to compete with full length DHFR mRNA for binding to DHFR protein (Ercikan Abali *et al*: Biochemistry *36*: 12317-12322, 1997), was placed at the 5' end of the gene coding the reporter protein, EGFP. Two sublines were constructed by stable transfection of C85 cells with either pEGFP-N1 (Clontech Takara) vector (pEGFP-C85 subline) or its derivative, containing the L-region at the 5' end of EGFP (L-pEGFP-C85 subline). Fluorescence intensity was studied, applying flow cytometry and fluorescence microscopy.

During 48 h exposure of C85 parental cells to 1 μM MTX, and the following 96 h growth in the regular medium, DHFR mRNA level remained unchanged. The enzyme protein level showed a slight (1.5-fold) increase, considered negligible and pointing to a general change in translation attributed to the outcome of MTX treatment, rather than specific influence of MTX on DHFR translation, as similar slight increase (1.2-fold) was also noted for EGFP level in pEGFP-C85 cells treated with the same dose of MTX. Enzyme specific activity dropped 2.2-fold in cells exposed to 1 μM MTX for 48 h with no further drop apparent following subsequent 96 h growth in regular medium. Compared to pEGFP-C85 cells, L-pEGFP-C85 cells demonstrated a considerable decrease in EGFP expression, assayed by fluorescence intensity studies, when both sublines were grown in the regular medium. However upon exposure to 1 μM MTX for 48 h, an 8-fold increase in EGFP fluorescence was observed for L-pEGFP-C85 cells, similar to a 5-fold increase found for pEGFP-C85 cells.

Conclusion: Analysis of DHFR expression in human adenocarcinoma C85 cells exposed to 1 μM MTX for 48 h demonstrated that the resulting G1 growth arrest is associated with a negligible increase in DHFR protein level

and ~2-fold decrease in DHFR specific activity, the latter apparently resulting from MTX tightly binding to DHFR molecule that persists in crude extract preparation. A distinct (8-fold) increase of EGFP fluorescence intensity in MTX-treated L-pEGFP-C85 cells confirmed the previously postulated suitability of employing EGFP as a reporter for imaging cellular response to MTX treatment, identified as G1 growth arrest. The present study demonstrates that the G1 phase cell cycle arrest of C85 cells, caused by MTX, does not involve any specific DHFR expression regulation.

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AN INTEGRATED APPROACH TO CANCER PROTEOMICS

Karin D. Rodland, Cheryl Baird, David Camp, Richard D. Smith and Richard Zangar

Pacific Northwest National Laboratory, Richland, WA 99352, USA

An exciting new trend in cancer research is the use of mass-spectrometry based proteomics to discover new protein-based biomarkers for early diagnosis, therapeutic response, and prognosis that promise to revolutionize management of patients with cancer. However, biomarker discovery efforts are impeded by analytical limitations in sensitivity and throughput of proteomic methods to define the complexity of the human plasma or tissue proteome. The most effective program to discover protein biomarkers requires team science, an integrated informatics platform, identification and quantitation of candidate biomarkers in disease tissue, and standardized procedures for analyzing candidate biomarkers in bodily fluids. We have developed a tiered approach to biomarker discovery that leverages unique instrumentation, including highly powerful mass spectrometers available at PNNL. Biomarker validation is achieved through custom designed, high-throughput, quantitative sandwich ELISA microarray analysis. Antibody pairs can be readily generated through the use of a novel yeast surface display system for recombinant antibodies developed at PNNL. These tools are currently being applied to problems in breast, ovarian, prostate and liver cancers, as well as other chronic diseases.

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PROTEOMICS STRATEGIES FOR MARKER DISCOVERY IN LUNG CANCER

M. Roeßler[1], M.-L. Hagmann[1], I. Lindner[1], J. Klöckner[1], T. Muley[2], F. Herth[2], P. Schnabel[3], J. Karl[1], B. Risse[1], M. Tacke[1], G. Pestlin[1] and P. Heiss[1]

[1]Roche Professional Diagnostics, Roche Diagnostics GmbH, Penzberg;
[2]Thoraxklinik Heidelberg, Heidelberg;
[3]Patholog. Institut, University Heidelberg, Heidelberg, Germany

Lung cancer is one of the most common malignancies in the Western population and 30% of all cancer deaths are caused by lung cancer, making it the number one killer among all cancer-related diseases. Today, less than 35% of lung cancer cases are diagnosed at an early stage and despite many recent achievements in imaging technologies, there is no appropriate screening tool available. To fill this diagnostic gap, accurate, non-invasive diagnostic markers in body fluids indicating early-stage disease would fulfil an urgent medical need. We are actively seeking such markers combining several proteomics technologies.

Despite many recent technological advances, especially in the field of mass spectrometry (MS), none of the currently available proteomics technologies offers the possibility to identify a comprehensive proteome of a given sample. Instead, it is necessary to combine several prefractionation methods with different MS technologies. Here, we present our approach to identify new potential marker candidates for lung cancer from tissue. This approach includes conventional two-dimensional gel electrophoresis (2-DE) and identification of proteins by MALDI-MS as well as liquid chromatography coupled to ESIMS. Additionally, samples were prefractionated by affinity chromatography and on preparative 1-DE gels.

After marker discovery programs applying proteomics technologies, it is of utmost importance to validate the findings with orthogonal methods. We apply immunoblot analyses, as well as immunohistochemistry to scrutinize the value of the identified marker candidates. Using these technologies it is possible to assess the potential of the identified marker candidates in a larger panel of lung cancer tissue samples as well as in other malignancies.

For the most promising candidates, highly sensitive immunoassays were developed to assess their presence in blood. Subsequently, we analyzed panels of well-characterized clinical blood samples of lung cancer patients and healthy controls. Data on the diagnostic performance of selected marker candidates will be presented.

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ASSOCIATION BETWEEN INFLAMMATORY MEDIATORS, D-DIMER LEVELS AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN PATIENTS WITH ADENOCARCINOMA

M. Roselli[1], V. Formica[1], F. Martini[2], I. Portarena[1], S. Mariotti[1], G. Del Monte[1], F. La Farina[2], I. Lucci[2], S. D'Angelo[2], P. Ferroni[2] and F. Guadagni[2]

[1]Medical Oncology, Department Internal Medicine, University of Rome "Tor Vergata", Rome;

[2]Laboratory of Thrombosis and Haemostasis, Department of Laboratory Medicine and Advanced Biotechnologies, IRCCS San Raffaele, Rome, Italy

Cancer cells release procoagulant activities responsible for thrombin generation and platelet activation. Platelet activation by thrombin, in turn, causes release of vascular endothelial growth factor (VEGF) a potent angiogenic factor essential for tumor growth and metastasis. It is also known that tumor cells and/or tumor-associated leukocytes may produce inflammatory cytokines, such as IL-6 and TNF-alpha. The release of inflammatory cytokines is also involved in activation of the fibrinolytic/ coagulation system. Based on these observations, a model has been proposed in which disturbance of the anti-inflammatory, anti-thrombotic state of endothelium by tumor cells would be responsible for platelet activation and release of angiogenesis-related factors. Thus, aim of this study was to investigate whether a correlation exists between inflammation, coagulation activation and VEGF release in patients with adenocarcinoma. Plasma VEGF, TNF-alpha, IL-6 and D-dimer levels were analyzed in 68 patients with lung (n=32, 63±8 years), colon (n=20, 62±17 years) or breast (n=16, 54±12 years) adenocarcinoma treated at the "Tor Vergata" Clinical Center. Thirty-four unrelated healthy controls (65±8 years) recruited from the same geographical area as the patients, served as controls. Written informed consent was obtained from each subject. The results obtained showed that median VEGF (30.4 *vs*. 24.3 pg/ml; $p=0.054$), TNF (2.5 *vs*. 0.3 pg/ml, $p<0.0001$), IL-6 (3.2 *vs*. 1.0 pg/ml, $p<0.0001$) and D-dimer levels (0.93 *vs*. 0.34 mg/ml, $p<0.0001$) were higher in patients than controls. Correlation analysis showed that plasma VEGF correlated with D-dimer (rho=0.24, $p<0.05$), TNF (rho=0.31, $p<0.01$) and IL-6 (rho=0.40, $p<0.001$) levels in adenocarcinoma patients. D-dimer, in turn, strongly correlated with both TNF (rho=0.57, $p<0.001$) and IL-6 (rho=0.60, $p=0.03$) levels. Positive VEGF levels (>41 pg/ml) were found in 63% of lung compared to 31% of breast, 20% of colon adenocarcinoma and 4% of controls ($p<0.0001$). Moreover, positive VEGF levels were associated to node involvement (60% *vs*. 30%, $p=0.02$) and positive D-dimer (67% *vs*. 25%, $p<0.001$), TNF (80% *vs*. 25%, $p<0.0001$) and IL-6 (78% *vs*. 27%, $p<0.01$) levels. Thus, to further analyze the relationship between VEGF and clinical and laboratory variables of adenocarcinoma patients, multiple regression analyses including age, gender, histological diagnosis, tumor size, node involvement, presence of distant metastasis, D-dimer, TNF and IL-6 levels were performed. Final models by stepwise analysis showed that TNF levels were independently associated to VEGF (beta=0.41, $p=0.003$) and D-dimer levels (beta=0.44, $p<0.001$).

The significant association found between plasma VEGF, TNF-alpha and D-dimer levels, suggests that the host inflammatory response to cancer cells, and/or their released products, as well as tumor-derived cytokines, could be responsible for an activation of the fibrinolytic/coagulation pathway and subsequent platelet VEGF release in patients with adenocarcinoma, especially of the lung.

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ROLE OF PHARMACEUTICAL EXCIPIENTS IN THE TAMOXIFENE ACTIVITY ON MCF-7 AND VERO CELL CULTURES

Tiziana Rossi[1], Valentina Iannuccelli[2], Gilberto Coppi[2], Elisa Bruni[1] and Giosué Baggio[1]

[1]Department of Biomedical Sciences, Section of Pharmacology, and
[2]Department of Pharmaceutical Sciences, University of Modena and Reggio Emilia, Modena, Italy

Pharmaceutical excipients are substances used in the commercial formulation of a drug to improve the efficacy of the active agent. Oral administration of the non-steroidal anti-estrogen tamoxifen is the treatment of choice for metastatic estrogen receptor-positive breast cancer. Tamoxifen shows a fairly good oral bioavailability combined with large inter-individual variations and few side-effects.

With the aim of improving tamoxifen bioavailability and achieving the active dosage at a tumour site for a long period, we studied the *in vitro* activity of a tamoxifen formulation in calcium alginate/chitosan microparticles. Moreover, the influence of alginate mannuronic/glucuronic ratio affecting a probable interaction with the cationic drug was evaluated by comparing two types of sodium alginate (from Kelco, France, with 62% mannuronic acid and 38% guluronic acid; and from Fluka, Switzerland, with 30% mannuronic acid and 70% guluronic acid) for the microparticle preparation. MCF-7 and Vero cells were cultured in Eagle's Minimum Essential Medium and treated with growing concentrations (from 10 to 100 μM/ml medium) of tamoxifen alone, microparticles loaded with tamoxifen, and microparticles alone as control. MTT test was performed on both the cultures.

The results observed after 24, 48 and 72 hours showed that both types of sodium alginate improved cell growth in a dose-dependent way. Tamoxifen LD_{50} values were similar (38 μM/ml) in Vero and MCF-7 cells only after a 24 h incubation time. The values we observed after 48 and 72 h showed a higher toxicity on Vero cells (LD_{50}=10 μM/ml) with respect to MCF-7 cells (25 μM/ml). Interesting results came from the comparison between the two types of microparticles loaded with tamoxifen. Microparticles released the drug after 48 hours and LD_{50} were significantly

changed both with respect to the drug alone and, in particular, to the type of alginate. When Kelco alginate was used, LD_{50} was enhanced: 25 μM/ml on Vero cultures and 48 μM/ml on MCF-7 cells; when Fluka alginate was used, LD_{50} was reduced: 25 μM/ml on Vero cells and 10 μM/ml on MCF-7 cells, thus showing a stronger activity of the formulation on both cells, but with a lower cytotoxicity on Vero cells.

In conclusion these preliminary results confirm that the microparticle formulation of tamoxifen can be useful in improving its bioavailability but we outline that the therapeutic dosages must be revised according to the excipients employed in the formulation since their link to the active compound can dramatically change both drug activity and toxicity.

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MORPHOLOGICAL AND MOLECULAR EVENTS AT THE ADVANCING EDGE OF COLORECTAL CARCINOMAS IN HUMANS

C.A. Rubio

Gastrointestinal and Liver Pathology Research Laboratory, Department of Pathology, Karolinska Institute and University Hospital, Stockholm, Sweden

The mechanisms whereby colorectal adenocarcinomas (CRC) invade the extracellular matrix (ECM) remain elusive. In a series of studies, we found at the growing edge of CRC, dilated neoplastic glands, some with a layer of flat tumor cells, and some lacking one or more groups of consecutively lining tumor cells (called glandular pores, GPs). Through the GPs, the retained glandular material was siphoned off directly into the juxtaposed ECM. The substance secreted by the tumor cells, rich in proteolytic enzymes, disrupted the paratumoral anatomy of the ECM. To remodel the defective glands, the malignant cells, proliferating from the tip of the free borders of the pores, invade the enzymatically disrupted matrix to achieve glandular continuity. We reported a similar sequence of events in colonic carcinomas in rats and in CRC in baboons. The sealing of the GPs permits the intraglandular accumulation of new proteolytic material, a mechanism that replicates a new wave of host invasion at the invading front, thus ensuring a stepwise but everlasting tumor progression in untreated patients. In recent studies, we found that laminin-5, an adhesion-migration macromolecule is frequently overexpressed in the tumor cells at the free ends of the GPs, indicating increased production of laminin-5. A close interaction between this adhesion-migration macromolecule, PG formation and the local progression of colonic carcinomas seems to occur. In more recent studies, we found that the flat tumor cells in the neoplastic glands at the advancing edge failed to express the proliferation marker Ki-67 but expressed mutated p53 protein. This paradoxical biological behaviour of tumor cells may be connected with the subsequent formation of glandular pores and strongly suggests that the arrested cell proliferation at the advancing tumor edge of CRC occurs independently of *p53* mutation. It is suggested that at the advancing tumor edge of CRCs, two independent molecular systems exist, one supervising cell proliferation and the other actively transferring the mutated p53 protein to daughter cells. These mechanisms of action would also explain the clinical appearance of metastasis many years after the surgical "curative" eradication of colorectal carcinomas, conveyed by "awakening" dormant cells.

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IMMUNE EVASION BY TUMOUR: LET'S GET BACK TO THE "ROOT" OF THE PROBLEM

Gaurisankar Sa[1], Tanya Das[1] and Charles S. Tannenbaum[2]

[1]Division of Molecular Medicine, Bose Institute, Kolkata, India;
[2]Department of Immunology, Cleveland Clinic, Cleveland, OH, USA

Immune dysfunction is well-documented in cancer patients, and likely contributes to tumour evasion. Often tumour targets and directs T-cell function to escape from immunosurveillance. This dysfunction includes loss of effector T-cells, type-2 cytokine bias and T-regulatory (T_{reg}) cell expansion. To account for immune escape by tumours, multiple mechanisms have been proposed including tumour-induced T-cell apoptosis. However, the molecules involved in these processes still remain controversial. We observed that tumour-shed various soluble factors play critical role in suppression of anti-tumour immunity. One of the factors was prostaglandin E_2, which severely affected cytokine receptor γ chain-mediated Jak-3/Stat-5a-signalling in T-cells resulting in T-cells demise. It was also observed that tumour-shed ganglioside-induced oxidative-stress perturbed NF-κB, thereby, leaving T-cells vulnerable to tumour-secreted TNF-α-induced apoptosis. Further investigation revealed that tumour burden up-regulated Treg populations that contributed to the decreased T-cell activation and Th-1 type of immune response.

The true therapeutic benefit of the use of plant products, especially acceptable dietary components, such as curcumin, has opened new horizons in cancer prevention and treatment. We observed that this yellow pigment of the spice turmeric not only regressed cancer, but also rejuvenated intrinsic defense machineries of the host, which become jeopardized during cancer. In fact, the entire immune dysfunction could be ameliorated by curcumin, indicating that this phytochemical has the ability to restore Jak-3/Stat-

5a- as well as NF-κB-signalling pathways in T-cells. Curcumin also blocked Treg cell augmentation *via* down-regulation of TGF-β in cancer cells and thus prevented tumour-induced loss of T-effector cells and reversed type-2 cytokine bias. The active component in the root of turmeric could re-educate the cellular signalling components and blocked immune evasion by tumor. Curcumin, therefore, may have a possible therapeutic potential in cancer patients in whom the immune capacity is affected not only by the disease itself, but also by the treatment measures. Thus, modern medical research seems to confirm the ancient wisdom that therapy of many diseases may reside in this inexpensive spice.

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EVALUATION OF SYNERGISTIC CYTOTOXICITY OF CO-ADMINISTRATION OF TAXOL AND ATORVASTATIN ON MDA-MB-468 CELL LINE

H. Sadeghi-Aliabadi[1], M. Minaiian[2] and M. Analoii[3]

[1]Department of Pharmaceutical Chemistry, [2]Department of Pharmacology, School of Pharmacy, [3]Isfahan Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Taxol is one of the cytotoxic drugs used for a wide range of cancers, but has some problems such as low permeability through the cell membrane and high price. Statins with low side-effects can potentially be used as cytotoxic agents acting with different mechanisms. In this study, synergic cytotoxic effects of co-administration of taxol and atorvastatin on the MDA-MB-468 cell line were evaluated. *Materials and Methods:* The MDA-MB-468 cells were cultured on RPMI medium. The cytotoxic activity of co-administration of taxol and atorvastatin were screened at 0.1, 1 and 2 μM and 0.25, 0.5, 1, 2, 4, 8, 16 and 32 μM, respectively. Cytotoxic activity of taxol 24 h after incubation of cells with atorvastatin was screened, using MTT method. *Results:* Atorvastatin had no effect on cells at a low concentration (0.25 and 0.5 μM), but growth was inhibited 100% at high concentrations (8, 16 and 32 μM). Synergic cytotoxic effect was observed when taxol was added 24 h after incubation of cells with atorvastatin. It has been shown that there is differential sensitivity of different cell lines to antitumor activity of statins. The results of the present study indicated that the MDA-MB-468 cell line has a low sensitivity to low concentrations of atorvastatin. *Conclusion:* Consistent with findings of other studies, the results of the present study indicated that addition of Taxol 24h after incubation of cells with atorvastatin had synergic cytotoxic effect on MDA-MB-468 by at least 20%. Consequently it may be possible to recommend *in vivo* co-administration of statins with cytotoxic drugs.

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CYTOTOXIC EVALUATION OF CO-ADMINISTRATION OF DOXORUBICIN WITH SIMVASTATIN AGAINST A HUMAN CANCER CELL LINE (HeLa) USING MTT ASSAY

H. Sadeghi-Aliabadi, M. Minaiyan and A. Dabestan

Isfahan Research Center of Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Doxorubicin is a broad spectrum antitumor antibiotic in the treatment of cancers. Its dose-dependent cardiotoxicity is the most serious side-effect causing drug withdrawal from hard chemotherapeutic regimens. Statins have been shown to be cytotoxic in concentrations that are higher than effective doses for hypercholesterolemia treatment (40 mg/day). Co-administration of statins and chemotherapeutic agents, such as doxorubicin was shown to be synergic. By this protocol, lower dose of cytotoxic agent can be used, and, hence, reduce the risk of cardiotoxicity. In this study, the cytotoxic effects of doxorubicin and simvastatin co-administration (in combination or alone) on HeLa tumor cell line were evaluated. *Materials and Methods:* HeLa cells were cultured in RPMI medium and cytotoxic effects of different concentrations of doxorubicin and simvastatin either alone or in combination were evaluated, using standard MTT assay. Briefly, after seeding cells (3×10^4 cell/ml) in 96-well plates and 24 h pre-incubation, compounds were added and plates were incubated (37˚C, 5% CO_2). After 72 h, 20 μl MTT solutions were added and incubated for another 3 h. Media were replaced with 150 μl of DMSO and absorbance was read at 540 nm using ELISA plate reader. Finally, cell survival was calculated as compared with control (100% viable cells). *Results:* Results showed that simvastatin at low concentration (0.25 μM) seems to be a growth stimulator (cell viability 112%, $p<0.05$), although at a concentration of 2 μM or more, cell viability was reduced by at least 20% ($p<0.5$). Doxorubicin (0.1, 1, 2 μM) reduced cell survival between 40 to 83%. Co-administration of two compounds at highest tested concentrations (2 μM) after 72 h killed 97% of cells ($p<0.05$). Incubation of cells with simvastatin after 24 h with doxorubicin seems to be a more effective protocol than the converse (14-76% cytotoxicity *versus* 12-61%). *Conclusion:* Between 3 tested protocols in these studies, co-administration of doxorubicin and simvastatin after 72 h incubation showed the highest cytotoxicity against HeLa cells. On the other hand, incubation of cells with doxorubicin, 24 h prior to addition of simvastatin was more effective. Co-administration of drugs for longer periods of time might help apoptosis more than each drug alone or incubation with one compound prior to other one.

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TUMOR SPECIFICITY AND TYPE OF CELL DEATH INDUCED IN ORAL SQUAMOUS CELL CARCINOMA CELL LINES BY NATURAL AND SYNTHETIC COMPOUNDS

Hiroshi Sakagami

Division of Pharmacology, Department of Diagnostic and Therapeutic Sciences, Meikai University School of Dentistry, Sakado, Saitama 350-0283, Japan

This is the review of our recent study on the tumor specificity and the type of cell death induced by more than 1,000 natural and synthetic compounds, using human normal oral cells (gingival fibroblast, pulp cell, periodontal ligament fibroblast) and human oral squamous cell carcinoma (OSCC) (HSC-2, HSC-3, HSC-4, Ca9-22, NA) and human glioblastoma (T98G, U87). Anthracyclines, such as doxorubicin, showed the highest tumor-specificity, but this was mostly, but not completely, related to the expression of *mdr1* mRNA expression in the target cells. Most of the lower molecular weight flavonoids and monomeric tannins showed little or no tumor specificity. On the other hand, oligomeric tannins showed slightly higher tumor specificity. There was no clear-cut relation between the tumor-specificity and apoptosis-inducing activity. α,β-Unsaturated ketones, such as 4,4-dimethyl-2-cyclopenten-1-one, trichloroacetylazulenes, α-trifluoromethyl acyloins, morphinone and codeinone, induced non-apoptotic cell death (little or no activation of caspase or internucleosomal DNA fragmentation) or autophagy (accompanying the formation of autophagosome, and occasionally secondary lysosome engulfing broken organelles). However, α,β-unsaturated ketones showed weak tumor specificity except for 3-arylidene-1-(4-nitrophenylmethylene)-3,4-dihydro-1*H*-naphthalen-2-ones that showed comparable tumor specificity with anthracyclines. Nocobactins, mycobactin-like siderophores, also showed higher tumor specificity, but their cytotoxicity was neutralized by chelation with iron. Five OSCC cell lines showed considerable variability in their sensitivity against antitumor agents. Inhibition of autophagy by epigallocatechin gallate or autophagy inhibitor enhanced apoptotic cell death in RAW264.7 macrophages, suggesting the interaction between apoptosis and autophagy.

A semi-empirical molecular orbital method may be useful to estimate the most stable structure of candidate compounds and explore compounds with greater tumor specificity.

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LARYNGEAL CARCINOMA IN MOSUL

AbdulMuhsen Y. Saleem and Muna Muneer

Mosul General Hospital, Mosul Medical College, Mosul, Iraq

Laryngeal carcinoma is a common malignant tumor among Asian races. Laryngeal carcinoma is regarded as the commonest upper aero-digestive tract cancer and the commonest head and neck cancer. More than 90% of laryngeal tumors are squamous cell carcinoma (SCC). Our aim was to present data about the incidence of various laryngeal carcinomas in Mosul after 1991 and to compare the results with previous studies. *Materials and Methods:* A retrospective study was carried out at the Oncology and Nuclear Medicine Hospital, Mosul Military Hospital, Al-Zahrawi Teaching Hospital and Alrahma Private Hospital, over an eight-year period from January 1994 - December 2002. Four hundred and twenty patients with laryngeal carcinoma were included in the study. Clinical data including age and gender of the patients, in addition to the histological reports from the case files were collected and analyzed. Statistical analysis using Z-test concerning male and female distributed cancer cases was carried out. *Results:* There were a total of 420 patients with laryngeal carcinoma, with a male to female ratio of 3.6:1. Of all cases, 97.2% were SCC and 1.4% adenocarcinoma. The age range was 36-85 years, with a mean age of 60 years. Z-test revealed a statistically significant gender difference (male *vs*. female, 78.3% *vs*. 21.7%, $p<0.01$) for laryngeal cancer. *Conclusion:* This study revealed a higher frequency of laryngeal cancer than that of previous studies performed in the same locality (Mosul) during the previous decade and this could be attributed to the general effect of the Gulf war in 1991 and the type of weapons used in it. There is a need for further studies in different parts of Iraq as to the cause of the increase in incidence of malignancy after the War of 1991.

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EVALUATION OF MLH1 AND *MSH2* GENE MUTATIONS IN A SUBSET OF IRANIAN FAMILIES WITH HEREDITARY NON-POLYPOSIS COLORECTAL CANCER (HNPCC)

Mansoor Salehi and Shahnaz Amani

Department of Genetics, Medical School, Isfahan University of Medical Sciences, Isfahan 81744/176, Iran

Hereditary non-polyposis colorectal cancer is the most common form of hereditary colorectal cancer, accounting for 5 to 10% of all colon carcinomas. It is inherited in an autosomal dominant mode and caused by germline mutations in mismatch repair genes (MMR), chiefly *MLH1* and *MSH2*. The lifetime risk of colon cancer in affected persons is 80%. Screening, prevention strategies and consequently treatment options will be improved by understanding of the genetic basis of this disorder. The aim of this study was to assess mutations in *MLH1* and *MSH2* genes in a subset of Iranian HNPCC patients.

Families fulfilling the Amsterdam criteria were selected as HNPCC families. Genomic DNA was extracted from the peripheral blood samples and germline mutations of *MLH1* and *MSH2* were detected by PCR-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing techniques. Germline mutations were found in 20 cases. The mutation rate in our study (62.5%) was higher in comparison with countries, such as Latvia or Taiwan. Of these mutations, 14 were found in *MLH1* and 6 in *MSH2* genes thus *MLH1* gene had a higher mutation rate than *MSH2*. Eighteen out of 20 detected mutations in our population were previously reported and two were novel.

Our results demonstrated that the mutation range, as well as genes involved in HNPCC is different from one region to other and characterizing mutations could be very helpful in diagnosis of the at-risk individuals.

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AN ORGANOTYPIC MYOMA MODEL FOR ANALYSING CARCINOMA INVASION

Sini Nurmenniemi[1], Pia Nyberg[1], Meeri Sutinen[1], Juha Risteli[2] and Tuula Salo[1,3]

[1]Institute of Dentistry and [2]Department of Clinical Chemistry, University of Oulu;
[3]Oulu University Hospital, Oulu, Finland

Traditionally human carcinoma invasion is studied using three-dimensional organotypic culture model using rat type I collagen gel disc with or without embedded human fibroblasts. Since carcinoma cells behaviour depends largely on their surroundings a more natural environment for cancer cell invasion studies should be created. In this study a novel organotypic culture model based on human leiomyoma tissue was established.

Commercial highly invasive human tongue squamous cell carcinoma cell line HSC-3 was cultured on top of bovine collagen gels and on human uterine leiomyoma discs for two weeks in the presence or absence of a gelatinase-specific peptide inhibitor CTT-2. Uterine leiomyoma tissues were obtained from the routine surgical treatments in the hospital and either processed immediately or frozen for later use. Organotypic culture media samples were collected every three days and were analyzed by radioimmunoassay for type I collagen, Western blotting of collagenases or using gelatin zymography. The organotypic tissue discs were formalin fixed and paraffin embedded sections were studied by immunostaining and in situ hybridization.

From myoma discs a wide variety of cell types, including myofibroblasts, endothelial and inflammatory cells were identified. In the extracellular matrix type I, III and IV collagen and laminins were present and synthesized by the invading carcinoma cells. Interestingly, in myoma model, particularly using frozen-thaw discs, carcinoma cells invaded and proliferated significantly more efficiently than in traditional collagen gels with fibroblasts. The invasive potential of the carcinoma cells and the inhibitory effect of CTT-peptide could also be analyzed from the culture media samples.

We have developed a novel human organotypic culture model based on human myoma tissue discs. This model is practical and convenient for analysing easily the effects of various invasion inhibiting substances on carcinoma cells.

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THE ACTIVATING ROLE OF SRC AND STAT3 ON HGF TRANSCRIPTION IN HUMAN BREAST CANCER CELLS

Michelle R. Sam[1], Bruce E. Elliott[2,3] and Christopher R. Mueller[2,3]

[1]Cancer Genomics Platform, Ontario Institute for Cancer Research, Toronto, Ontario, Canada;
[2]Department of Pathology and Molecular Medicine, and
[3]Division of Cancer Biology and Genetics, Queen's Cancer Research Institute, Kingston, Ontario, Canada

Hepatocyte growth factor (HGF) participates in normal mammary development through tightly regulated paracrine signaling. Aberrant regulation of HGF and its receptor Met is thought to contribute to tumorigenesis, and overexpression of both proteins is frequently observed in breast cancer. Active Src and Stat3 are mediators of HGF/Met signaling, and previous studies have demonstrated that cooperative Src/Stat3 signaling activates *HGF* transcription in mouse mammary carcinoma cells. Characterization of the *HGF* promoter led to the identification of a Stat3 consensus site located at -95nt which was shown to be required for full *HGF* transcriptional activity. To extend this work in the context of human breast cancer, activated Src and Stat3 were overexpressed in human epithelial cell lines and their signaling effects on HGF activation assessed.

Results from this study demonstrate that human breast cells provide a permissive environment for HGF transactivation mediated by activated Src/Stat3 interaction. Endogenous protein expression patterns were also characterized for the proteins involved in HGF/Met signaling across a variety of epithelial cell lines. Furthermore, use of Stat3 shRNA approaches to knockdown Stat3 expression show that Stat3 mediates *HGF* transcription through interactions at the -95nt site on the *HGF* promoter. The generation and use of inducible Stat3 fusion proteins also allowed for the independent evaluation of Stat3 dimerization, activation and transcriptional regulation of the HGF/Met signaling pathway. Results from this study support the suggested model of a cooperative activating role of Src/Stat3

signaling, and their participation in the formation of an HGF autocrine loop. This may in turn lead to sustained activation of the HGF/Met signaling pathway thereby promoting an oncogenic phenotype in human mammary epithelial cells. Better understanding of these proteins and their interactions within the signal transduction pathway can further aid in the target and design of therapeutic approaches to breast cancer treatment.

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A NOVEL RADIOIMMUNOCONJUGATE FOR POSSIBLE USE IN THE DETECTION OF HNSCC

Karl Sandström[1], Marika Nestor[1], Tomas Ekberg[1], Mats Engström[1], Matti Anniko[1] and Hans Lundqvist[2]

[1]Unit of Otolaryngology and Head and Neck Surgery, Department of Surgical Sciences, Uppsala University, Akademiska Sjukhuset, Uppsala;
[2]Unit of Biomedical Radiation Sciences, Department of Oncology, Radiology, and Clinical Immunology, Uppsala University, Uppsala, Sweden

The presence or absence of lymph node and distant metastases has a major impact on the preference of treatment in patients with head and neck squamous cell carcinoma (HNSCC). Radioimmunodiagnosis could offer a more specific and sensitive diagnostic method than those available today. Our aim was to label cMAb U36 with ^{111}In and study the biodistribution of the labelled compound in HNSCC xenografts and selected normal organs of nude mice. We also wanted to evaluate imaging of HNSCC using the same compound in a planar gamma camera. *Materials and Methods:* In this study we used the chimeric monoclonal antibody U36 (cMAb U36) targeting CD44v6, expressed in many squamous cell carcinomas, and the humanized antibody huA33 targeting A33, expressed in colorectal carcinomas, as a negative control. The antibodies were labeled with the Auger electron emitter Indium-111 (^{111}In), using the chelator CHXA''-DTPA. *In vitro* binding characteristics of the ^{111}In labeled cMAbU36 and huA33 conjugates were made with an immunoreactivity assay and binding studies in order to determine the specificity of the labeled antibodies and the presence of the antigens CD44v6 and A33 on SCC9 cells. Biodistribution and tumor imaging studies were conducted after intravenous injection of the radiopharmaceuticals in nude mice bearing HNSCC xenografts expressing CD44v6. *Results:* The antibody tolerated labeling conditions very well. The immunoreactive fraction was very high (>95%), and blocking experiments verified that the binding was CD44v6-specific. *In vivo* results demonstrated a promising biodistribution, with tumor being the only tissue to clearly accumulate radioactivity with time. All organs had substantially lower radioactivity than the blood. The blood-to-tumor ratio of cMAb U36 increased continuously during the study, from 0.24±0.10, 6 h p.i., to 4.45±1, 172 h p.i. The targeting specificity of cMAb U36 was further supported by the significantly (p<0.038) higher uptake 72 h p.i. compared to huA33. The static images of the tumor xenografts showed good visualization of the tumors. *Conclusion:* We have produced a novel radioimmunoconjugate targeting CD44v6, for possible use in radioimmunodiagnosis of HNSCC. The conjugate was evaluated both *in vitro* and *in vivo*, demonstrating no adverse effects from labeling, and a favorable biodistribution. The planar gamma camera images showed good visualization of the tumors. The specific, high, and accumulating blood-to-tumor ratio indicated a favorable retention of the conjugate, a promising property for future imaging studies.

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POSSIBLE ROLE OF DIFFERENTIAL METALLOPROTEINASE 1 EXPRESSION IN SIGNET RING CELL AND INTESTINAL COLORECTAL CARCINOMA HISTOTYPES

R. Sanguedolce[1], D. Cabibi[2], A. Martorana[2], F. Aragona[2] and A. Calascibetta[1]

Dipartimenti di [1]Scienze Farmacologiche e di [2]Anatomia Patologica, Policlinico, Facoltà di Medicina e Chirurgia, Università degli Studi di Palermo, Italy

Background: Signet ring cell colorectal carcinoma (SRCC) pure is an infrequent and highly malignant variant of colorectal cancer, while this histological component is present in 30% of all colorectal carcinomas. In our previous studies, we compared the E-cadherin, β-catenin, fibronectin, Ki-67 and thymidylate synthase (TS) expression of SRCC with those of the intestinal type of colorectal carcinoma (ICRC) to try to show the pathogenesis, aggressiveness and low 5-fluorouracil (5-FU) responsiveness of these tumours. We found that all SRCCs had very low levels of all markers and were in the post-mitotic phase of the cell cycle. To understand their high metastatic capability we assessed the SRCC expression of matrix metalloproteinase-1 (MMP1), a proteolytic enzyme, suspected to play an important role in the progression of various types of cancer and compared it to the ICRC one. *Materials and Methods:* We tested MMP1 expression immunohistochemically on formalin-fixed, paraffin-embedded samples of 32 SRCC and 70 ICRC. Differences in the distribution of the study variables, and associations between variables were assessed by means of the χ^2 test. *Results:* SRCC showed a high expression of MMP1 over all the invasion front of the tumour and in the neoplastic embolus, rather than the ICRC (p<0.001). *Conclusion:* As MMPs seem to play important role in tumour invasion and metastasis, recently they have gained attention as targets for new anticancer therapy strategies. MMP

inhibitors have been shown to prevent tumour spread both *in vitro* and *in vivo* and to inhibit tumour angiogenesis and some of them are being developed for clinical use. In this study we demonstrated that SRCCs showed a different MMP1 expression pattern compared to ICRC, for this reason an anti-MMP therapy should be advisable in these patients because of their poor prognosis.

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MULTI-SCALE MODELLING OF THE IMMUNE SYSTEM: APPLICATIONS TO CANCER IMMUNOTHERAPY

C.E. Sansom[1], A. Shepherd[1], V. Brusic[2,3], S. Motta[4], P.L. Lollini[5], M.-P. Lefranc[6] and D.S. Moss[1]

[1]School of Crystallography, Birkbeck College, London WC1E 7HX, UK;
[2]Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA;
[3]LCAFS, University of Queensland, Brisbane, Australia;
[4]Department of Mathematics and Informatics, University of Catania;
[5]Department of Hematology and Oncological Science, University of Bologna, Italy;
[6]IMGT®, Institut de Génétique Humaine, CNRS, Montpellier, France

Vaccination and monoclonal antibody (mAb) immunotherapy are becoming increasingly important and effective strategies for the treatment of some types of cancer (1), with over 1,850 clinical trials for vaccines and immunotherapy currently listed in the international registry ClinicalTrials.gov. Many of these studies were based on computer modelling. Immunoinformatics is a growing sub-discipline, bridging bioinformatics and immunology. It is concerned with modeling and computational analysis of the human immune response at the molecular, cellular and system levels.

The best-established area of immunoinformatics is probably molecular level modeling, which has clinical implications in predicting prognosis in CLL (2) and evaluating humanized antibodies for immunogenicity (3). Predictions of peptide-MHC binding have contributed to the development of therapeutic vaccines, although this approach cannot be used in isolation (4). At the system level, the *in silico* mouse model has been used to predict the optimum vaccination schedule for preventing mammary tumor development in ERBB2/HER-2/NEU transgenic mice (5).

Recently, we have been combining these approaches by developing and implementing an integrated model of the human immune system in the ImmunoGrid project (http://igrid-ext.cryst.bbk.ac.uk/immunogrid/site/index.html), funded through Framework 6. An enormous amount of data related to the immune response at different levels is being collected and curated in a repository and fed back into our simulators. Many of the calculations required are computationally intensive and are distributed between systems that our partners have access to using Grid technology. We are now seeking funding to develop this basic science into novel computational tools that will be accessible and useful in the clinic. In this presentation, both our current applications of immunoinformatics to cancer vaccine development, and our future plans will be described.

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CANCER COMMUNICATION: SHARING BENEFITS, EXPLAINING RISKS

C.E. Sansom

School of Crystallography, Birkbeck College, London WC1E 7HX, UK

The subject of cancer is always newsworthy and often emotionally charged. Researchers and clinicians working in this area may be relatively fortunate in their funding environment, but they have to cope with the glare of publicity. Cancer researchers, like all scientists, inhabit a world of slow advances and statistical uncertainty, but it is not unusual for such incremental advances to be reported by journalists in glowing terms, involving clichés such as “boffin” and “miracle cure”. And in the age of the Internet-taught “expert patient”, some patients will rapidly become aware of these “breakthroughs” and demand novel treatments, sometimes before they have even been licensed.

It is important that both basic and clinical cancer researchers take the issue of communication seriously. Unlike most scientists, who have to battle for media attention, cancer specialists will often be addressing a large audience who are not scientifically literate – who may lack the training to interpret figures about, for example, the risks of contracting certain cancers or the benefits of a particular drug. People are prepared to accept risks most if the hazard is familiar, if there is an associated benefit, and if they have some control over whether they are exposed to it (1). This may go some way to explain why, when the connection between smoking and cancer is so widely recognized, about 13 million UK adults still smoke. It is also difficult for people to understand the complex linkages between genetics

and cancer. Those who may be at risk cannot always judge the benefits (and the reverse) of genetic tests. Within the professional cancer community, too, all cannot readily understand each other's jargon, and even researchers and clinicians in different sub-disciplines can seem to be speaking different languages.

The traditional "deficit model" (2) – which would mean a one-way flow of technical information from researchers and oncology specialists to general practitioners, and thence to patients – cannot be the whole story. Generalist physicians, who lack specialist oncology training but are closest to individual patients' experiences (3), can develop communication skills that the specialists often lack. In this presentation I will discuss the need for, and benefits of, improving communication between different cancer professionals and between those professionals and those they seek to serve, and discuss how best to foster this important skill.

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THE ROLE OF ABC TRANSPORTERS IN RESISTANCE TO ANTICANCER TYROSINE KINASE INHIBITORS

Balázs Sarkadi, Csilla Özvegy-Laczka and Csilla Hegedűs

Membrane Research Group; Hungarian Academy of Sciences, Semmelweis University, 1113 Budapest, Dioszegi u. 64, Hungary

The three major types of multidrug-resistance (MDR) transporter proteins in humans include members of the ABCB, the ABCC, and the ABCG subfamily. These pumps recognize a wide range of drug substrates, mostly hydrophobic compounds, but also a variety of amphipathic anions and cations. MDR-ABC proteins play an important role in cancer drug resistance, but also in the absorption, distribution, metabolism and toxicity (ADME-tox) of several pharmaceutical agents.

The ABCG2 transporter provides tissue protection against numerous toxic compounds and accounts for a multidrug-resistant phenotype in cancer cells. It has been documented by us and others that ABCG2 displays a high affinity interaction with several clinically effective small molecule tyrosine kinase inhibitors. These include Iressa® (Gefitinib), an inhibitor of EGF-receptor dependent signalling, and Imatinib (Glivec®) which inhibits the Bcr-Abl fusion protein, the molecular basis of chronic myeloid leukemia (CML).

In this study, we investigated the interaction of ABCG2 with two second-generation Bcr-Abl inhibitors already in clinical trials, Nilotinib and Bosutinib. We examined the effects of these compounds on the ATPase activity of the human ABCG2, on Hoechst 33342 dye extrusion and on the survival of Bcr-Abl+ K562 cells, overexpressing the ABCG2 protein (K562/ABCG2). Our data indicate that the novel Bcr-Abl inhibitors have major differences in their interaction profiles with ABCG2. While Nilotinib is a high affinity substrate and at higher concentrations is an effective inhibitor of the transporter, Bosutinib does not significantly interact with ABCG2 at therapeutically relevant concentrations. These findings may provide important information regarding the anticancer effects and ADME-Tox properties of novel tyrosine kinase inhibitors.

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ASTROCYTE ELEVATED GENE-1 (AEG-1): A NOVEL REGULATOR OF HEPATOCELLULAR CARCINOMA

Devanand Sarkar

Department of Human and Molecular Genetics,Massey Cancer Center,Virginia Commonwealth University, Richmond, VA, USA

Hepatocellular carcinoma (HCC) is a highly aggressive vascular cancer characterized by diverse etiology, activation of multiple signal transduction pathways and mutations in a plethora of genes with no effective treatment. We presently document that expression of Astrocyte Elevated Gene-1 (AEG-1) is extremely low in human hepatocytes and it gradually increases with the stages and grades of human HCC by analyzing Affymetrix microarray data from 144 HCC patients and immunostaining of tissue microarray from 109 HCC patients. Stable overexpression of AEG-1 converts non-tumorigenic human HCC cells into highly aggressive vascular tumors and inhibition of AEG-1 abrogates tumorigenesis by highly aggressive HCC cells. Microarray analysis identifies that AEG-1 modulates expression of genes associated with invasion, metastasis, chemoresistance,

angiogenesis and senescence, all intimately relevant to HCC pathogenesis. AEG-1 activates Wnt/β-catenin signaling *via* ERK42/44 activation and up-regulates LEF-1/TCF-1, the ultimate executor of Wnt pathway. Inhibition studies demonstrate that activation of Wnt signaling plays a key event in mediating AEG-1 function. AEG-1 also activates NF-κB pathway that might be relevant to chronic inflammatory changes preceding HCC development. These pleiotrophic modulation of multiple signaling pathways and gene expression place AEG-1 as a central molecule regulating diverse aspects of HCC pathogenesis. Thus targeted inhibition of AEG-1 might lead to the shutdown of key elemental characteristics of HCC and be an effective therapeutic strategy for HCC.

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THE ROLE OF MAPK SIGNALING PATHWAY IN MALIGNANT BONE AND SOFT TISSUE TUMORS

Kanji Sasaki, Toshiaki Hitora, Osamu Nakamura, Yoji Kawaguchi and Tetuji Yamamoto

Kagawa University, Orthopedic Surgery, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa-ken, 761-0701, Japan

Background: Raf-1 and MEK1/2 which is an essential serine/threonine kinase, consist a downstream of the central signal transduction mediator Ras in the MAPK (mitogen-activated protein kinase) signaling pathway (RAF/MEK/ERK pathway). Furthermore, various growth factors such as VEGF stimulate Ras and activate the MAPK signaling pathway. Therapeutics targeting anti-VEGF, Raf-1 and MEK are currently undergoing clinical evaluation on some human malignancies. We consider that MAPK signaling pathway play an important role in malignant bone and soft tissue tumors. We therefore examined the expression of VEGF, Raf-1 and MEK 1/2 genes and the inhibitory effects of MAPK signaling pathway. *Materials and Methods: Specimens, cell lines and reagent*. We used the 20 specimens of human malignant bone and soft tissue tumors, which had been clinically and historogically diagnosed. Three human osteosarcoma cell lines (KHOS, KTHOS and MG-63) and 3 human malignant fibrous histocytoma (MFH) cell lines (GBS-1, Nara-F and Nara-H) were used. All cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, St. Louis,MO) supplemented with 10% fetal bovine serum (FBS;Sigma-Aldrich). The cells were routinely maintained at 37˚C in a humidified 5% CO_2 atmosphere. Bevacizumab, a humanized anti-VEGF monoclonal antibody was purchased from Genentech. GW5074, a specific Raf-1 inhibitor was purchased from Sigma-Aldrich. U0126, a selective MEK1/2 inhibitor, was purchased from Promega. *mRNA expression of VEGF, Raf-1 and MEK1/2*. Total RNAs were eluted by selective binding to a silica-gel-based membrane using an RNeasy Mini Kit® (QIAGEN Inc., Valencia, CA). Reverse transcription of RNA into cDNA was performed by using Reverse Transcription System (Promega, Madison, WI). Raf-1 and MEK1/2 mRNA expression were examined by reverse transcription (RT-)PCR. After PCR amplification, 8-μl aliquots of the PCR products were electrophoresed in a 2% agarose gel, followed by ethidium bromide dye. *The inhibitory effect of Bevacizumab and GW5074 and U0126*. Cell proliferation was assayed using the MTS assay (CellTiter 96® Aqueous One Solution Cell Proliferation Assay; Promega, Madison, WI). Cells were seeded in 96-well cell culture plates. After 48 hours (h), the medium was renewed and Bevacizumab or GW5074 or U0126 was added at the indicated concentrations. After 12, 24, 48h, the optical density was measured. The percent viability of each well was calculated. *Results: mRNA expression of VEGF, Raf-1 and MEK 1/2*. The mRNA of Raf-1 and MEK 1/2 was strongly expressed in all 20 specimens and all 6 cell lines. The mRNA of VEGF was expressed in all 6 cell lines. *The effect of Bevacizumab*. Bevacizumab inhibited cell proliferation of all 6 cell lines at the viability of 80%. *The effect of MAPK signaling pathway inhibitors*. GW5074 and U0126 inhibited cell proliferation of all 6 cell lines in a dose- and time-dependent manner. 10 μM GW5074 and 50 μM U0126 inhibited cell proliferation by 50% or less. *Discussion:* The MAPK pathway is very important as a target of molecular therapy, because it is specific in malignant tumors. In our study, the mRNA of Raf-1 and MEK1/2 were strongly expressed in all the specimens of malignant bone and soft tissue tumors, and all osteosarcoma and MFH cell lines. MAPK inhibitors GW5074 and U0126 showed an inhibitory effect on cell proliferation. These results suggest that the MAPK signaling pathway exists in all malignant bone and soft tissue tumors and plays an important role in the proliferation of osteosarcoma and MFH. In our study, all cell lines exhibited VEGF-gene expression, and inhibition of VEGF also decreased cell proliferation. These results suggest that VEGF stimulate not only the vascular growth but also the MAPK signaling pathway. Thus we suggest that both VEGF, and the downstream of Ras have a relationship with the control of cell proliferation in osteosarcoma and MFH.

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LONG-RANGE EPIGENETIC SILENCING AND CPG ISLAND METHYLATOR PHENOTYPE (CIMP) – A KEY MECHANISM IN COLORECTAL CARCINOGENESIS?

Pawel Karpinski[1], Nikolaus Blin[2] and Maria M. Sasiadek[1]

[1]Department of Genetics, Wroclaw Medical University, Poland;
[2]Division of Molecular Genetics, Institute of Human Genetics, University of Tuebingen, Germany

Colorectal cancer (CRC) belongs to the group of most frequent cancers in humans. Among all cases of CRC, about 75% are sporadic, 5-10% hereditary, while 15% are familial. To date, two main molecular pathways have been revealed as being critical in CRC: i) specific for familial adenomatous polyposis (FAP), which is characterized by loss of function of *APC* gene and thereafter chromosomal instability; and ii) specific for hereditary nonpolyposis colon cancer (HNPCC), which is characterized by loss of function of one of the mutator genes (usually *MLH1* or *MSH2*), followed by microsatellite instability. These pathways are also revealed as specific for 85% and 15% of sporadic CRC, respectively.

The involvement of epigenetic alternations in molecular etiology of CRC has been discussed for more then 20 years. Thereafter, much of our current knowledge on epigenetic alterations in cancerogenesis comes from studies on CRC. Two epigenetic phenomenon connected with CpG island methylation are observed in CRC: i) CpG island methylator phenotype (CIMP) which is characterized by exceptionally high frequency of methylated CpG islands specifically localized in promoter regions of a number of genes; and ii) recently discovered long-range epigenetic silencing (LRES), which is associated with DNA/histone methylation. LRES can span large chromosomal domains and involves broad heterochromatin formation accompanied by hypermethylation of clusters of contiguous CpG islands within the region, demonstrating that DNA hypermethylation can span larger chromosomal regions and can lead to silencing of flanking, unmethylated genes. Recently, two LRES domains have been reported in CRC. One at 2q14.2 by Frigola *et al*. (2006) and a second localized at 3p22 by Hitchins *et al*. (2007).

It is also noteworthy that both examples of LRES are associated with transcriptional silencing of genes that that are known to play an important role in carcinogenesis *e.g*. *hMLH1, EN1* (Wnt signaling pathway), *GLI2* (sonic hedgehog signaling pathway), *DLEC1* (tumor suppressor) and *CTDSPL* (tumor suppressor). Moreover, aberrant methylation which expands thorough tumor progression is potentially reversible, opening therefore new perspectives in cancer diagnosis and therapy.

Altogether these results suggest the interrelationship between MSI, CIMP and LRES in the etiology of CRC.

We investigated whether these two phenotypes (CIMP and LRES) are interrelated in CRCs. Both CIMP and methylation of *MLH1* gene as well as of three CpG islands (*EN1, SCTR* and *INHBB*, corresponding to three distinct clusters along 2q14.2) were determined by methylation-specific PCR while *BRAF* V600E mutation was sought by mutated allele-specific amplification PCR. A total of 88% of cases showed hypermethylation of at least one of the three CpG islands along 2q.14.2. The average number of these sites showing methylation in CIMP+ tumors was 2.21 compared to 1.22 for CIMP– individuals (p=3.5×10^{-8}, Mann-Whitney test). Moreover, all CIMP+ tumors showed hypermethylation of at least one of these loci in contrast to CIMP– tumors, where 16% of samples remained unmethylated. The mean number of simultaneously hypermethylated CpG islands at 2q14.2 differed significantly for CIMP– and CIMP+ tumors, suggesting different effects of domain silencing in this region. Given that the number of hypermethylated loci at 2q14.2 likely affects the range of silenced flanking genes, a high frequency of simultaneous hypermethylation of three CpG islands (*EN1, SCTR* and *INHBB*) may have potential influence on specific characteristics of CIMP+ CRCs.

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E2F7 EXHIBITS BOTH TUMOUR SUPPRESSIVE AND ONCOGENIC ACTIVITIES IN HUMAN KERATINOCYTES

Liliana Endo-Munoz[1], Alison Dahler[1], Danny Rickwood[1], Anthony Griffin[2], Geoffrey Strutton[3] and Nicholas Saunders[1]

[1]Diamantina Institute for Cancer, Immunology and Metabolic Medicine, University of Queensland, Princess Alexandra Hospital, Queensland;
Departments of [2]Surgery and [3]Pathology, Princess Alexandra Hospital, Queensland, Australia

We have previously shown that inhibition of E2F activity is essential for the initiation of squamous differentiation (1, 2). For this reason, we postulated that endogenous inhibitors of E2F may exist that are involved in the initiation of squamous differentiation. In the present study, we examined the potential role of E2F7 in regulating E2F-dependent functions in human keratinocytes (proliferation, differentiation and apoptosis). We found that E2F7b was the most highly expressed splice variant of E2F7, whose expression was restricted primarily to proliferating keratinocytes. In a series of reporter assays, we were able to show that E2F7 could inhibit E2F1-dependent proliferation markers and could sensitise keratinocytes to subsequent differentiation stimuli. Based on these data, we propose that E2F7b is likely to be a physiologically-relevant regulator of the initiation of squamous differentiation. We next went on to show that E2F7b was a potent suppressor of E2F1-induced keratinocyte apoptosis. Combined, these data suggest that the antiproliferative, pro-differentiative activity of E2F7 is consistent with a tumour suppressor function, whilst the antiapoptotic activity is consistent with that of an oncogene. This situation is the opposite to that reported for E2F1. This proposition takes on more significance when combined with our observation that E2F7b and E2F1 are highly expressed (10- to 100-fold) in human cutaneous squamous cell carcinomas (SCC). This would suggest that the overexpression of E2F7 could contribute to SCC formation.

These data would also suggest that the inhibition of E2F7 could be a novel way to induce apoptosis or sensitise SCCs to cytotoxic drugs.

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TREATMENT OF METASTATIC MELANOMA PATIENTS WITH TUMOR INFILTRATING LYMPHOCYTES: CLINICAL DEVELOPMENT

Jacob Schachter, Michal J. Besser, Ronny Shapira-Frommer, Avraham J. Treves, Dov Zippel, Orit Itzhaki, Ester Schallmach, Adva Kubi, Naama Zabari, Bruria Shalmon-Zifroni, Izhar Hardan, Eran Segal, Raphael Catane and Arnon Nagler

Ella Institute for Treatment and Research of Melanoma and Skin Cancer, Sheba Medical Center, Tel Hashomer 52621, Israel

Background and Purpose: After the Surgery Branch/NCI (1), the "Ella Institute" at the Sheba Medical Center in Israel, is the second center worldwide that successfully adopted the tumor-infiltrating-lymphocytes (TIL) technology, which consists of the adoptive transfer of large numbers of selected anti-tumor reactive TIL to lympho-depleted melanoma patients. This protocol requires the generation of multiple individual TIL cultures from one patient and the *in vitro* selection of TIL cultures, which specifically secrete IFNγ after co-culture with melanoma cells. The establishment of multiple, relatively homogenic TIL cultures demands often prolonged culture times and many fail the selection criteria. Preclinical animal models and clinical studies have shown that lymphocytes that spend less time in culture tend to have longer telomeres and less-differentiated phenotypes, which can translate into objective response as well as persistence *in vivo* (2, 3). Furthermore, extended culture time leads to reduced TIL heterogeneity. Considering these facts a modified clinical protocol using "young" TIL, with short-term cultures was applied. This technology furthermore does not require the IFNγ selection criteria, which impact is not fully verified yet. *Clinical Study:* The eligibility criteria include stage IV melanoma patients, previously treated with chemotherapy and IL-2, a good performance status (PS-0,1) and no brain metastasis. *Clinical Protocol:* Patients receive non-myeloablative lympho-depleting chemotherapy with cyclophosphamide (60 mg/kg) on days -7 and -6 and fludarabine (25 mg/m^2) on days -5 to -1 prior to TIL infusion (day 0), followed by bolus high-dose IL-2 (720 000IU/kg every 8 h) to a maximum of 15 doses. *Results:* Until today, 20 melanoma patients have been enrolled to the protocol. Twelve were treated by the selected TIL protocol and 8 were treated by the "young" TIL protocol. The preliminary results are encouraging. No treatment-related death or major toxicity (grade 3 or 4, besides from expected pancytopenia) were observed. All patients had neutropenic fever. *Conclusion:* We demonstrate that admission of this novel protocol is feasible. The preliminary results are encouraging but further clinical studies are necessary in order to prove the clinical benefit of this program.

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REFINING NON-HUMAN PRIMATE CANCER MODELS: BEHAVIORAL FACTORS

Steven J. Schapiro

Department of Veterinary Sciences, The University of Texas M.D. Anderson Cancer Center, 650 Cool Water Dr., Bastrop, TX 78602, USA

The use of non-human primates as models in cancer research is increasing in frequency and importance. Given the social and cognitive complexity of the species of non-human primates likely to be utilized in cancer-related investigations, it is of the utmost importance to refine the techniques used to manage and handle these animals. While physiological measures are often used to assess effects of manipulations in many experiments, behavioral factors that may influence these physiological measures are too frequently overlooked. We have demonstrated that behavioral factors, such as social housing conditions, the techniques used to anesthetize the animals, the techniques used to attain blood samples, and the relocation of animals from one facility to another, can all significantly alter physiological readouts in a number of non-human primate species. Our data indicate that not every behavioral factor will result in statistically significant changes in all relevant physiological measures, but many factors will alter variables vital for assessing disease state. One of the primary values of understanding the effects of behavioral factors is to refine management, handling, and research techniques so as to minimize intersubject variability due to potential behavioral confounds, especially in a Good Laboratory Practices environment. By implementing behavioral refinements that minimize variability between subjects, critical, and expensive, experiments involving non-human primate subjects can be conducted with fewer subjects without a reduction in statistical power.

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NEOADJUVANT CHEMOTHERAPY IN BARRETT'S CARCINOMA – PROGNOSIS AND RESPONSE PREDICTION

M. Schauer, K.P. Janssen, B. Holzmann, M. Feith, J. Theisen and W.T. Knoefel

Klinikum rechts der Isar, Technische Universität Munich, Germany

It has been shown that the incidence of esophageal adenocarcinoma is increasing at an alarming rate in Western countries. More than 50% of patients present with locally advanced disease. In these patients, preoperative chemotherapy appears to increase the chance for a curative resection and enhance survival in responding patients. Unfortunately Barrett's carcinoma shows a very heterogenous behaviour in progression and response to neoadjuvant chemotherapy. Response occurs in only 50% of patients after chemotherapy with cisplatin, 5-fluorouracil or leucovorin. If patient outcome could be predicted accurately, treatments could be tailored individually to avoid chemotherapeutic induced side-effects in non-responding patients. Prediction of the final response seems to be possible by measuring the metabolic response by PET scans at the beginning and after two weeks of chemotherapy. Differentiation of responders from non-responders even before starting chemotherapy might be possible in the future using microarray technology and immunohistology in endoscopically obtained biopsies of the tumor.

We found a pattern of 86 genes that were at least 2-fold differentially regulated comparing responding and non-responding adenocarcinomas of the esophagus. The predominant genes encoded for the regulation of the cell cycle, transduction, translation, cell–cell interaction, cytoskeleton and the signal transduction. The strongest difference was seen for the ephrin B3 receptor, a tyrosin kinase that is already known in other tumor entities for its role in cell growth, migration and invasion. The receptor is negatively controlled by the β-catenin/TCF-complex. Ephrin B3 signaling couples cell contraction with cell-to-cell adhesion by promoting the recruitment of E-cadherin to the membrane.

In conclusion our results suggest that it could be possible to characterize patients responding to chemotherapy before starting the treatment using customized microarray analysis. Ephrin B3 receptor may serve as an important new biological tumor marker for local invasion and chemotherapy response or even as a therapeutically relevant target. Further examinations are necessary to evaluate these results and a possible therapeutic utility.

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CROSSING THE BARRIER: FROM INTRAVENOUS TO EFFECTIVE ORAL FORMULATIONS OF CHEMOTHERAPY

Jan H.M. Schellens

The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, NL;
Science Faculty, University Utrecht, Utrecht, the Netherlands

Oral bioavailability of many anticancer drugs is poor and highly variable. This is a major impediment to the development of new generation drugs in oncology, particularly those requiring a chronic treatment schedule, a.o. the tyrosine kinase inhibitors and oral angiogenesis inhibitors. Limited bioavailability is mainly due to: 1) cytochrome P450 (CYP, especially CYP3A) activity in gut wall and liver, and 2) drug transporters, such as P-gp and breast cancer resistance protein (BCRP, ABCG2) in gut wall and liver. Shared substrate drugs are affected by the combined activity of these systems. Preclinical *in vitro* and *in vivo* models have in many cases been poorly predictive for oral drug uptake in patients because of a.o. interspecies differences in CYP drug metabolism and intestinal drug-transporting systems. For this reason, we have developed novel and in part humanized systems that allow reliable translation of preclinical results to the clinic (Drug Metab Dispos *33*: 892-895, 2005; J Clin Invest *117*: 3583-3592, 2007).

Our previous work, also using P-gp knockout (KO) mice, already showed that P-gp has a major effect on the oral bioavailability of several drugs and that blockers of P-gp can drastically improve oral bioavailability of the important anticancer drug paclitaxel and other drugs in mice and humans (Cell *77*: 491, 1994; PNAS *94*: 2031, 1997; Lancet *352*: 285, 1998; J Clin Oncol *19*: 1160-1166, 2001). This work revealed, however, that apart from P-gp, other drug-transporting systems and CYP effects also determine overall oral drug uptake. Indeed, we demonstrated that blockade of ABCG2 profoundly increased the oral bioavailability of topotecan in mice and man (J Natl Cancer Inst *92*: 1651-1656, 2000; J Clin Oncol *20*: 2943-2950, 2002).

In addition, we demonstrated in four phase II activity studies that 'boosting' of the oral bioavailability of the widely applied anticancer drugs paclitaxel and docetaxel by the P-gp and CYP3A inhibitor cyclosporin A (CsA) resulted in high antitumor activity in advanced breast, non-small cell lung (NSCLC) and gastric cancer (J Clin Oncol *20*: 4508-4516, 2002; reviewed by us in TIPS *27*: 17-24, 2006). More recently we demonstrated that in order to enable oral therapy with docetaxel, the boosting agent CsA could well be replaced by the more effective and selective CYP3A

inhibitor ritonavir; this concept has been proven in our mouse models (Cancer Res *62*: 6158-6164, 2002) and in patients (BCPT, 2007; Br J Clin Pharmacol, 2008, in press).

For the preclinical and clinical studies the intravenous (*i.v.*) solutions of paclitaxel and docetaxel have been used, as these drugs are only available for *i.v.* use. However, these formulations are highly unsuitable for further oral clinical development for practical and safety reasons and due to limited pharmaceutical stability. For this reason, we have developed a range of novel capsule formulations of docetaxel and paclitaxel that need urgently to be tested in patients with cancer. Preclinical pharmaceutical tests performed by us reveal excellent dissolution of active drug.

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TRANSGENIC STRATEGIES TO STUDY HUMAN DISEASES

Johannes Schenkel

German Cancer Research Centre and University, Heidelberg, Germany

Transgenic mice exhibit an enormous scientific potential for many purposes, including serving as a specific *in vivo* model for human diseases. Different techniques are used to generate genetically modified animals harbouring a gain or a loss of function. The number of transgenic mice is increasing rapidly. Small populations, continued danger of loss, the need to keep these mutants in stock and frequent interchange of transgenic mice between different facilities are some of the major challenges for laboratory animal scientists when dealing with these unique mutants.

Two transgenic mouse models for human diseases will be presented: *The regulation of the human papilloma virus (HPV) 11:* About 100 HPVs; are described, some of them are associated with malignant growth, others with benign proliferations. Subsequently it is of paramount interest to understand their regulation, but these viruses show a high host-specificity. To study HPV-regulation in a human-free *in vivo*-system transgenic mice were generated carrying a reporter gene under control of the key regulatory element (URR) of HPV11. A developmentally regulated, inducible, and specific transcription was observed, but the expression pattern in the original target (skin) was not satisfying in this host and virus free system. To be closer to the *in vivo* conditions, a transgenic mouse model was established expressing the virus activity influencing protein: HPV11-E2. The transgenic expression of the E2 did not lead to phenotypic changes. E2/URR11 double-transgenic mice are already bred to study the HPV11 regulation in more detail. *Signal transduction in a mouse model for neuro-degenerative diseases:* Neurodegenerative diseases often result in an (excessive) neuronal cell death, leading often to a large, non-reversible injury. However, the molecular mechanisms or pathways responsible are not fully understood.

The c-Jun transcription factor plays a crucial role in promoting apoptosis, inhibition of its activation might reduce the size of cell death and thus increase functional outcome. Several genetically modified mice influencing c-Jun and its pathways at various steps and were investigated in a model for experimental stroke and other neurodegenerative diseases. Our data suggest that attenuation rather than a complete block of c-Jun action appears more promising for therapy of stroke.

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ORIGIN AND DEVELOPMENT OF GLIOMAS IN RELATION WITH PROGNOSIS AND THERAPIES

D. Schiffer, M. Mellai, V. Caldera, L. Annovazzi and E. Andreoli

Neuro-bio-oncology Center, Policlinico di Monza, University of Turin, Vercelli, Italy

The origin of gliomas is discussed in the light of the classic experiments of transplacental induction of tumors by nitrosoureas in the rat and their development from germinative and subventricular zones. Today this origin became a general knowledge supported by an extensive literature on stem cells, progenitors and precursors with the recognition of the existence of brain tumor stem cells. Two concepts are fundamental: the relationship between factors regulating cytogenesis and the genetic alterations which characterize tumor transformation and the importance of epigenetic events. The possibility that differentiated cells may revert to a stem cell-like status and that undifferentiated cells may acquire the same status, assume a particular significance in tumor progression. All these considerations affect histological diagnosis, prognosis and therapy of gliomas, including their cell resistance. The problem becomes more complicated not only if other characteristics of tumor malignancy are considered, such as cell motility and migration, but also the recently acquired knowledge that stem cells from SVZ or hemispheric adult cells of the astrocytic lineage can move toward the tumor or that non tumor adult astrocytes can be recruited into the tumor. To the observations concerning the different molecular pathways leading to tumor transformation, those based on the recent use of siRNA and those carried out on the apoptosis/autophagy system must be added.

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DOWNSTREAM AKT REGULATION OF PROLIFERATION, APOPTOSIS AND AUTOPHAGY IN GLIOBLASTOMAS

D. Schiffer[1], L. Annovazzi[1], V. Caldera[1], M. Mellai[1], L. Tessitore[2] and G. Valente[3]

[1]Neuro-bio-oncology Center, Policlinico di Monza, University of Turin, Vercelli; [2]DISCAF and [3]Clinical and Experimental Medical Department, University of East Piedmont, Novara, Italy

PI3/AKT pathway plays an important role in the process of transformation of gliomas. Under the control of growth factors, it terminates by regulating proliferation and apoptosis. Two steps in the pathway, STAT3 and mTOR (target for Rapamycin) have been recognized as possible targets for tumor therapy, but in human gliomas their correlation with proliferation and apoptosis are not completely known. In 64 gliomas STAT3 and mTOR have been investigated by molecular genetics, immunohistochemistry and Western blotting procedures in relation with EGFR, EGFRvIII, PTEN, AKT, Caspase-3, PARP.1, S6, Beclin 1 and Ki.67/MIB.1. STAT3, mTOR and AKT together with S6 increased with malignancy grade, but only S6 correlated with the proliferation index, whereas there was a good correlation of EGFR/EGFRvIII with AKT. Both STAT3 and mTOR correlated with AKT and in the terminal part of the pathway S6 indicated SK1 as an important step toward proliferation. No correlation was found of STAT3 and mTOR with apoptosis, maybe for technical difficulties of its demonstration in tumor tissues. On the contrary, both were inversely correlated with Beclin 1, in line with the knowledge that autophagy is not activated in malignant gliomas. The rationale for the identification of STAT3 and mTOR as therapy targets seems to be well-grounded in human gliomas.

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ELECTRON EMISSION FROM GENISTEIN IN RELATIONSHIP TO ITS ANTICANCER PROPERTIES

N. Getoff, H. Schittl and R.M. Quint

Section of Radiation Biology, Department of Nutrition Sciences, The University of Vienna, Althanstr. 14, UZA II, A-1090 Vienna, Austria

Genistein (GEN; 4',5,7-Trihydroxyisoflavone) is a phytohormone with biological properties similar to those of estradiol. It has been established that GEN shows antiangiogenetic and antitumor effects *in vivo* (1). Very recently it was observed that under the influence of oxidizing (OH, $O_2^{\bullet-}$), as well as reducing free radicals (e^-_{aq}, H *etc.*), GEN features a strongly expressed antitumor ability *in vitro* and acts synergistically on mitomycin C (2).

On the other hand it has been demonstrated that sexual hormones such as 17β-estradiol, progesterone (3), as well as testosterone (4) can eject electrons from their excited singlet state in polar media. Under certain biological conditions this ability of the hormones can result into metabolites involved in the initiation of breast and prostate cancer, respectively.

The present work reports the electron ejection from in singlet state excited GEN, initiated by monochromatic UV-light (λ=254 nm; 4.85 eV/hν) in aqueous solution (pH~7.4 at 37˚C).

Comparing the molar extinction coefficients (ε in $L.mol^{-1}.cm^{-1}$) at the absorption maxima (270 nm) it was proved that GEN has a tendency to form "associates" (labile GEN-complexes) at concentrations above 5×10^{-6} $mol.L^{-1}$. On the other hand GEN possesses high reactivity towards e^-_{aq}, $k(e^-_{aq} + GEN) = 6.2\times10^9$ $L.mol^{-1}.s^{-1}$ (5). As a result of this fact solvated electrons could not be found using 1×10^{-5} $mol.L^{-1}$ GEN. By application of 5×10^{-5}, 7.5×10^{-5} and 1×10^{-4} $mol.L^{-1}$ GEN at pH~7.5 were obtained: $Q(e^-_{aq}) = 2.4\times10^{-3}$, 1.6×10^{-3} and 1.3×10^{-3}, respectively. On the other hand using air-free, aqueous 5×10^{-5} mol.L-1 GEN at 30˚, 37˚ and 45˚C, increasing quantum-yields ($Q(e^-_{aq}) = 1.9\times10^{-3}$, 2.4×10^{-3}, 4.8×10^{-3}, respectively) were observed, indicating that the $Q(e^-_{aq})$-yield also depends on temperature.

The biological consequences of the bifunctional property of GEN: electron emission and electron consumption in respect to cancer are discussed. Its bifunctional ability enables GEN to communicate with other systems in the organism.

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3 Getoff *N et al*: Photo-induced electron emission from 17β-estradiol and progesterone and possible biological consequences. J Photochem Photobiol, B: Biology *92*: 38-41, 2008.

4 Getoff N *et al*: Electron emission from photo-excited testosterone in water-ethanol solution. J Photochem Photobiol., B: Biology: submitted.

5 Cai Z *et al*: Interaction of hydrated electron with dietary flavonoids and phenolic acids: Rate constants and transient spectra studied by pulse radiolysis. Free Radic Biol Med *27*: 822-829, 1999.

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TISSUE MICROARRAYS ARE RELIABLE TOOLS FOR THE CLINICOPATHOLOGICAL CHARACTERIZATION OF LUNG CANCER TISSUE

Lars Henning Schmidt[1], Stefan Biesterfeld[2], Andreas Kümmel[1], Andreas Faldum[3], Martin Sebastian[1], Christian Taube[1], Roland Buhl[1] and Rainer Wiewrodt[1]

[1]Pulmonary Division, III. Medical Clinic, [2]Institute of Pathology and [3]Institute of Medical Biostatistics, Epidemiology and Informatics, Johannes Gutenberg-University, 55101 Mainz, Germany

Background: The advantage of tissue microarray (TMA) is its ability to efficiently analyze large numbers of tissue specimens in a methodologically uniform way. The reliability of TMAs especially with regard to clinicopathological characterizations when compared to conventional immunohistochemistry (IHC) was evaluated. *Materials and Methods:* Seventy two embedded tissue sections from lung cancer patients were stained with monoclonal antibodies against the tumor-associated markers TA-MUC1 and Lewis Y. Three representative cores of every tumor were embedded in a paraffin array multiblock. The IHC was evaluated by the immunoreactive score (IRS). *Results:* The data for the TMA IHC and the conventional IHC were concordant (kappa ≥80%) for both markers. Likewise, discordance (McNemar's test) was low and sensitivity and specificity were above 80% for both markers. In the samples with high positive expression, the concordance increased (kappa ≥90%) discordance disappeared (McNemar p=1.0) and sensitivity and specificity increased above 90% for both markers. Using Cox regression models, all the clinicopathological dependencies were equivalent for both techniques and both markers. *Conclusion:* Immunohistochemistry with tissue microarrays is valid and provides results equivalent to conventional immunohistochemistry with respect to expression patterns and clinicopathological characterizations.

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BCL-2, BCL-XL, AND PP32/PHAPI IN RESECTED NON-SMALL CELL LUNG CANCER PATIENTS

Lars Henning Schmidt[1], Andreas Kümmel[1], Martin Schuler M.[2], Stefan Biesterfeld[3], Andreas Faldum[4], Martin Sebastian[1], Christian Taube[1], Roland Buhl[1] and Rainer Wiewrodt[1]

[1]Pulmonary Division, III. Medical Clinic, [3]Institute of Pathology, and [4]Institute of Medical Biostatistics, Epidemiology and Informatics, Johannes Gutenberg-University Medical Center, 55101 Mainz; [2]Department of Medicine (Cancer Research), West German Cancer Center, University Hospital Essen, 45122 Essen, Germany

Deregulation of apoptosis signaling is commonly found in cancer cells. Some studies suggest that overexpression of the anti-apoptotic proteins Bcl-2 and Bcl-xL in non-small cell lung cancer (NSCLC) is associated with adverse prognosis. This is thought to result from their function as cell death suppressors and resistance factors in the mitochondrial pathway. Another important factor in this pathway is pp32/PHAPI, as it seems to sensitize NSCLC cells to apoptotic stimuli and thus permitting an improved outcome in patients following chemotherapy for advanced NSCLC. However, contradicting observations have been described by other groups. Against this background, we have evaluated the prognostic effect of Bcl-2, Bcl-xL, and pp32/PHAPI expression in tumor samples from 87 surgically resected NSCLC patients using tissue microarray immunohistochemistry. Bcl-xL expression was immunohistochemically detectable in 20 cases (23%), Bcl-2 in 25 cases (29%) and pp32/PHAPI in 78 cases (90%). Using Cox proportional hazards models, Bcl-xL and pp32/PHAPI expression, normal lung function and early stage were identified as independent favorable prognostic factors in NSCLC patients. This contrasted the established role of anti-apoptotic proteins as resistance factors in patients receiving chemotherapy. Interestingly, impairment of lung function was associated with Bcl-2 expression, which may suggest a biological involvement of Bcl-2 family proteins in the pathogenesis of chronic obstructive pulmonary disease, which commonly precedes the development of NSCLC.

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FIBROBLAST-DERIVED TUMOR MARKERS: FROM PROTEASES TO microRNA

Boye Schnack Nielsen

Exiqon A/S, Diagnostic Product Development, Skelstedet 16, 2950 Vedbaek, Denmark

Cancer cell invasion is a multicellular process involving a heterogeneous group of stromal cells in addition to the cancer cells themselves. The fibroblasts constitute a basic component among stromal cells that contribute to matrix remodeling, synthesis and degradation. In most adenocarcinomas, the fibroblasts are found as activated and myo-differentiated fibroblasts, myofibroblast. Myofibroblasts are found in benign, pre-invasive (*in situ*) and invasive carcinomas, and thus, are not an indication of malignancy itself. However, myofibroblasts start expressing novel matrix degrading proteases, such as matrix metalloprotease 13 (MMP13), and urokinase plasminogen activator (uPA), during the transition from noninvasive (DCIS) to invasive breast cancer, and therefore are likely to promote the transition to invasion by degrading basement membrane collagen and laminin. We have recently identified some microRNAs in cancer associated fibroblasts. MicroRNAs represent a new and relatively uncharacterized group of 20-24nt long RNA sequences that show great promise as biomarkers in cancer. They can be detected in tissue sections by in situ hybridization using locked nucleic acid (LNA)-modified oligos. Although little is known about the precise functional aspects of microRNA, they have already

been shown to be important regulators that influence major biological processes, including tumorigenesis.

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MULTIPLEXED PROTEIN ANALYSIS APPLIED TO BREAST CANCER BIOPSIES

Nicole Schneiderhan-Marra[1], Georg Sauer[2], Helmut Deissler[2] and Thomas Joos[1]

[1]NMI, Natural and Medical Sciences Institute at the University of Tuebingen, Markwiesenstraße 55, 72770 Reutlingen;
[2]Department of Obstetrics and Gynaecology, University of Ulm Medical School, Frauensteige 14, 89075 Ulm, Germany

One of the most promising approaches in cancer research is the identification of biomarkers that allow defining of patient subgroups that will benefit from specific treatment schemes. Potential biomarkers or pattern of biomarkers can be identified using a target-driven approach. Protein microarrays can be applied to simultaneously quantify numerous target proteins from minimal amounts of sample material.

We have used the bead-based Luminex technology to develop multiplexed sandwich immunoassays to analyze a set of 54 pre-defined breast cancer relevant proteins: 113 lysates of shock-frozen large core needle biopsies were analysed using these assays. Validation of the bead-based immunoassays was carried out by comparing HER2 and ER protein expression with data obtained by the corresponding immunohistochemical routine assessment.

Concordance of the generated protein expression data sets with results of conventional immunohistochemical staining was demonstrated for the expression of the estrogen receptor and of HER 2. Furthermore, a set of five proteins was identified, which allows the prediction of nodal involvement. Further methodological refinement of multiparametric protein analyses and associated clinical studies will most likely lead to parameter sets which define subgroups of patients who will benefit from specific treatment regimens.

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CONTEMPORARY TREATMENT OF PRIMARY ADVANCED EPITHELIAL OVARIAN CANCER

Susanne E. Schneuber, Heinz S. Scholz, Vesna Bjelic-Radisic and Raimund Winter

University of Graz, Department of Obstetrics and Gynecology, Auenbruggerplatz 14, A - 8036 Graz, Austria

Ovarian cancer continues to be one of the major causes of death from cancer among women in the Western world. The majority of patients present with stage III or IV disease and the advanced stage of disease at the time of diagnosis is the main factor contributing to the overall poor prognosis.

In this review we give an overview on screening, FIGO staging, survival rates, prognostic factors and on contemporary treatment in early and advanced ovarian cancer.

In particular we report on our experience with multivisceral cytoreductive surgery in advanced epithelial ovarian cancer. Additionally, we outline the role of adjuvant chemotherapy as a standard of care and the role of neoadjuvant chemotherapy, intraperitoneal chemotherapy and hyperthermic intraperitoneal chemotherapy (HIPEC) as possible prospective standards of care in patients with ovarian cancer.

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MIGRATION STIMULATING FACTOR (MSF) AS A MEDIATOR OF EPITHELIAL-STROMAL INTERACTIONS IN HUMAN TUMOURS

Ana M. Schor, Ian R. Ellis, Sarah J. Jones, Stephane Perrier, Go Ohe and Seth S. Schor

Unit of Cell and Molecular Biology, The Dental School, University of Dundee, Dundee DD1 4HR, UK

Migration stimulating factor (MSF) is a 70 kDa truncated isoform of fibronectin. It is generated from a foreshortened pre-message produced by the transcriptional read through of the intron separating fibronectin exons III-1a and III-1b.

MSF is an oncofetal regulatory molecule constitutively expressed by three cell populations (epithelial, fibroblast and endothelial) during fetal development, not expressed in the majority of healthy adult tissues, and re-expressed by both tumour and tumour-associated stromal cells in common human cancers. MSF is also systemically re-expressed by skin fibroblasts derived from distant, uninvolved sites in breast cancer patients. Recent data indicate that the level of MSF expression by carcinoma cells and tumour-associated fibroblasts is inversely related to survival in patients with oral and breast tumours.

Cultured fibroblasts maintain the MSF production status of their tissue of origin. We have recently developed experimental protocols that enable us to induce MSF-producing fibroblasts to switch-off MSF expression and, conversely, to induce normal adult fibroblasts to switch-on MSF. These changes in MSF expression are hereditable (persistent in the absence of further treatment), but completely reversible, suggesting that the switch-on and-off of MSF expression is controlled by epigenetic mechanisms.

Recombinant MSF displays a number of bioactivities of relevance to cancer development and metastasis, including stimulation of cell invasion through 3D matrices, hyaluronan synthesis, angiogenesis and proteolytic activity. Two MSF isoforms have been cloned, and inhibitors produced by epithelial and endothelial cells have been identified. Our data

indicate that MSF acts as a paracrine and autocrine factor on three cell populations (epithelial, endothelial and fibroblasts) and its bioactivity is modulated by the type of isoform produced, the presence of other soluble factors and the nature of the extracellular matrix in contact with the cells.

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MYTHS AND FACTS IN THE SURGICAL MANAGEMENT

Guido Schumacher

Department of General-, Visceral-, and Transplantation Surgery, Charité Campus Virchow Klinikum, Berlin, Germany

Indication, surgical procedures, and postoperative management has been developed during the last centuries to reach the modern standards of patient care. Since the introduction of evidence based medicine, many clinical trials have been performed to prove or disprove effects of certain steps of surgical management. However, many of the actual diagnostic and therapeutic steps have never been proven, even though their necessity and usefulness is considered to be essential for a successful treatment. A major reason may be the opinion of individual specialists who internationally report on their personal experiences as given facts. This has a particular impact if a leading specialist is able to teach other specialists who then spread certain techniques in patient care and surgery. The value of personal experience appears to be valuable, since the disease and presents with a very large individual variability, which makes clinical trials often difficult and cause criticism. Examples of non-evidence-based diagnostic and therapeutic steps are the use of certain drainages, certain anastomotic techniques, strategies for the treatment of anastomotic leakages and peritonitis and others. We will discuss important steps of the perioperative management with emphasis on the actual evidence, which is based on clinical trials or personal experiences of specialists.

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PRO AND CONS FOR RADICAL SURGERY IN OVARIAN CANCER?

Jalid Sehouli, Guelten Oskay-Oezcelik, Guido Schumacher and Werner Lichtenegger

Department of Obstetrics and Gynecology, Charite Medical University, Germany

Despite maximal cytoreduction and adjuvant chemotherapy with paclitaxel and carboplatin, a large number of patients with epithelial ovarian cancer will suffer from tumor recurrence within 3 years after their initial diagnosis.

In contrast to the situation of patients with primary ovarian cancer the value of surgery in the salvage setting is controversially discussed.

Two prognostic groups should be differentiated: patients with platinum-sensitive ovarian cancer and those with platinum-resistant ovarian cancer. The indication for surgery is palliation to control clinical symptoms (*e.g.* bowel obstruction) and to increase the progression free survival and overall survival. Nevertheless, the pros and cons arguments base on retrospective data, only. Based on the available evidence from the literature and a large series of data from our center, all surgical aspects are discussed in detail.

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ROLE OF BLM AND 53BP1 DURING HOMOLOGOUS RECOMBINATION

Vivek Tripathi, Sarabpreet Kaur, Tirunelvely Nagarjuna and Sagar Sengupta

National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110067, India

Mutations in BLM helicase causes Bloom Syndrome, characterized by predisposition to all forms of cancer. Hence lack of BLM and its interactions with its partners can lead to deregulation of RAD51-mediated homologous recombination. We have demonstrated that BLM, signal transducer 53BP1 and RAD51 form a trimeric complex both *in vitro* and *in vivo*. Phosphorylation in specific domains of the three proteins mediated this interaction. Presence of BLM enhanced the interaction between 53BP1 and RAD51. The interactions between the three proteins had functional consequences. Lack of 53BP1 decreased cell survival and enhanced chromosomal aberration after replication arrest. 53BP1 exhibited both BLM-dependent and independent anti-recombinogenic functions. Both BLM and 53BP1 abrogated endogenous RAD51 foci formation and disrupted RAD51 polymerization. The disruption of RAD51 polymerization was abrogated when non-binding or phosphorylation-deficient mutants of BLM and 53BP1 were used. As a result, loss of BLM and 53BP1 synergistically enhanced stress-dependent HR. These results provide evidence regarding the cooperation between two signal transducers, BLM and 53BP1, during maintenance of genomic integrity.

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ELECTROCHEMOTHERAPY – CLINICAL EFFECTIVENESS ON CUTANEOUS AND SUBCUTANEOUS TUMOR NODULES AND ITS VASCULAR DISRUPTING EFFECT

Gregor Sersa

Institute of Oncology Ljubljana, Zaloška 2, SI-1000 Ljubljana, Slovenia

Electrochemotherapy is a local drug delivery approach aimed at treatment with palliative intent of cutaneous and subcutaneous tumor nodules of different histology. Electrochemotherapy, *via* cell permeabilizing electric pulses potentiates the cytotoxicity of non-permeant or poorly permeant anticancer drugs with high intrinsic cytotoxicity, such as bleomycin or cisplatin, at the site of electric pulse application.

Physicochemical basis of this therapy allows prediction that electrochemotherapy has good antitumor effect on all tumor types, which was demonstrated in several clinical studies. The results of the clinical trial demonstrated that an objective response rate of 85% (73.7% complete response rate) was achieved on the electrochemotherapy-treated tumor nodules, regardless of tumor histology, and drug used (bleomycin, cisplatin) or route of its administration (intravenous, intratumoral). Most frequently it is used in the treatment of multiple cutaneous metastases of melanoma when they cannot be surgically excised due to their number or localization. In such cases, long-term remission up to several years can be obtained. Electrochemotherapy can also be used as a cytoreductive treatment before surgical resection in an organ-sparing attempt.

Bleeding skin melanoma metastases are a common but difficult management problem. Many patients are in poor physical condition, and therefore electrochemotherapy is very convenient, as it is quick, outpatient-based and effective. The effectiveness on bleeding tumors is due to vascular disrupting effect of electrochemotherapy. The underlying mechanism of blood flow reduction and vascular disrupting action has been studied extensively. In electrochemotherapy, where endothelial cells are in direct contact with the chemotherapeutic drugs, they undergo cell death due to the increased drug uptake.

Electrochemotherapy is now a clinically acknowledged method for the treatment of cutaneous and subcutaneous tumors. Its advantages are high effectiveness on tumors with different histology, simple application, minimal side-effects and the possibility of effective repetitive treatment.

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MOLECULAR ANALYSIS OF BREAST CANCER METASTASIS TO BONE IN AN "ALL HUMAN" NOD/SCID MOUSE MODEL

W. Yang, P. Lam, Y. Amemiya, H. Kahn, A. Yee, C. Holloway and A. Seth

Sunnybrook Research Institute, University of Toronto, Toronto, ON, Canada

Background: Bone is the most common site of metastasis in breast cancer, affecting up to 80% of women with advanced disease, leading to bone complications or skeletal-related events. Current studies on the bone metastasis process have been hampered by the lack of preclinical models to evaluate novel therapeutics and to study the biology of the disease. *Materials and Methods:* To create an "all human" mouse model that explicitly investigate the bone metastatic behavior of human breast tumors, we first engrafted human bone fragments into the both flanks of NOD/SCID mice and subsequently transplanted primary human breast tumor under only left flanks of the hu-bone NOD/SCID mice. *Results:* We found that engrafted human bone remains functional for more than 20 weeks of implantation and that approximately 30% of primary breast tumors survived and generated tumors when placed into hu-bone NOD/SCID mice. After performing serial re-transplantation experiments using primary breast tumors, we observed metastasis to the opposite tumor-free human bone fragments and some host tissues including kidney, lung and lymph nodes. Interestingly, none of the human breast tumors metastasized to the mouse skeleton, providing evidence that species-species osteotropism is essential for bone metastasis. Gene expression profiles using whole genome microarrays were generated from engrafted patient breast tumors to identify genes that correlate with growth and metastasis. Two groups of clinically significant genes are emerging from our ongoing results. One group represents genes associated with the future biological behavior of the original patient tumors. They are found by comparing gene levels between patient breast tumors that did and did not metastasize in the hu-bone NOD/SCID model. A second set of genes associated with the metastatic phenotype, but not necessarily predictive of that state, are revealed by comparing gene expression levels between the original patient tumors and the tumors that form in the human bone implants and mouse tissues. In the first group, relative to the non-metastatic tumor, 36 genes with significantly altered expression pattern were found in the primary tumors that subsequently metastasized in the mouse model. In the second group, relative to the original patient tumors, 205 genes were found to be significantly altered in tumors that grew in first human bone implants. Of these, 183 were also found in tumors that metastasized to second initially tumor-free human bone implants and in lung metastases. Two of these include *CSF1R* which is up-regulated and *IGFBP7* which is down-regulated. Both *IGFBP-7* and *CSF1R* are important in mammary gland development and have been implicated in breast carcinogenesis and associated with poor outcome in breast cancer patients. *Conclusion:* By using the "all human" NOD/SCID mouse model we have identified many potential targets that are up- or down-regulated in bone metastatic breast tumor samples. We anticipate that our study will help in developing breast cancer biomarkers for patients that are at high risk for bone metastasis and will be valuable for clinical diagnosis and treatment.

This work was supported by the Canadian Breast Cancer Research Alliance special program grant on metastasis.

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THE ANTICARCINOGENIC ACTIVITY OF VITAMIN D WITH AND WITHOUT IONIZING RADIATION

Shraga Shany

Department of Clinical Biochemistry, Ben-Gurion University of the Negev, Beer Sheva, 84105, Israel

Recent data suggest that besides its major role in mineral homeostasis, the active metabolite of vitamin D, 1.25-dihydroxyvitamin-D_3 ($1.25(OH)_2D_3$) has an anti-carcinogenic activity. However, its therapeutic use is limited, since it causes hypercalcemia in the concentrations effective for cell growth inhibition. In order to overcome this limitation, the present study have checked two attitudes: i) The possible use of the less-calcemic vitamin D synthetic analog 1.25-dihydroxy,16-ene,24-oxo,vitamin D_3 (*JK-1624-3*) and to compare its anti-carcinogenic activity, *in vitro*, with that of the native vitamin D active metabolite, $1.25(OH)_2D_3$. ii) The possible use of lower concentrations of $1.25(OH)_2D_3$ in combinations with other anti-carcinogenic agents, in order to get an additive or even synergistic effects. Similarly, the combination of vitamin D treatment with ionizing radiation was tested *in vitro* on prostate cancer cells.

Radiotherapy, by itself, is a common effective anticarcinogenic treatment. However, this treatment is accompanied by many complications. The possibility that pretreatment with vitamin D and other anticarcinogenic agents will potentiate the therapeutic effect of radiation was assessed in the present study. Incubation of cancer cells (HL-60, HT-29, LNCaP cell lines) with JK-1624-3 revealed a significant inhibition of cell proliferation, similar to that obtained by $1.25(OH)_2D_3$. These effects were found to be dose- and time-dependent. These vitamin D compounds cause a significant induction of cell cycle arrest in the G1-phase, as determined by FACS analysis. Incubations of *HL-60* cells with either $1.25(OH)_2D_3$, or the synthetic analog, revealed a very significant differentiation to monocyte-like cells, as expressed by the increased levels of CD-11b and CD-14 in the treated cells. The combined treatment of vitamin D, valproic acid and radiation was tested on androgen-refractory prostate cancer cell line *DU-145*. While radiation alone caused 30.6% cell growth inhibition, pretreatment with $1.25(OH)_2D_3$, valproic acid, or both agents, revealed cell growth inhibition of 46.4%, 83.0% and 87.9%, respectively. Cell cycle analysis showed that radiation following the combined treatment resulted in an increase in apoptosis and cell accumulation mainly in the S-phase of cell cycle.

In conclusion, the present results confirm the anticarcinogenic activity of the native vitamin D active metabolite. These results emphasize the possible therapeutic use of less calcemic vitamin D analogs, such as JK-1624-3. The combined pretreatment potentiates the effect of radiation and therefore may allow the use of lower concentrations of $1.25(OH)_2D_3$, on one hand, and lower radiation doses, on the other hand. This combined treatment may be more effective with fewer side-effects.

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COMBINED PRETREATMENT OF VITAMIN D AND HISTONE DEACETYLASE INHIBITOR ENHANCES SENSITIVITY TO RADIATION IN PROSTATE CANCER CELLS

Vladimir Gavrilov[1], Yaron Leibovich[2], Olga Belochitski[2], Samuel Ariad[2] and Shraga Shany[1]

Departments of [1]Clinical Biochemistry and [2]Oncology, Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Radiotherapy is known to be an effective treatment of prostate cancer (PCa). However, many complications are involved including rectal bleeding, erectile dysfunction and urinary incontinence. Therefore, it is important to develop PCa-sensitizing pretreatments that could potentiate the therapeutic effect of radiation, allow the use of lower radiation doses and limit the side-effects.

Toward these aims, we suggest combining the anticarcinogenic vitamin D active metabolite, $1,25(OH)_2D_3$, and sodium valproate (an antiepileptic drug that inhibits histone deacetylase activity) in order to sensitize PCa cells to radiation. The rationale of this notion is based on preclinical studies reporting increased efficiency of radiation after $1,25(OH)_2D_3$ or sodium valproate pretreatment (Dunlap *et al.*, 2003; Camphausen *et al.*, 2005). We evaluated the effect of the suggested treatment on the androgen-refractory PCa cell line DU145. Cancer cells were grown in RPMI-1640 medium containing 10% FCS. DU145 cells were pretreated for three days with 100 nM $1,25(OH)_2D_3$ or 1 mM sodium valproate, or their combination. After that, PCa cells were irradiated with a dose of 4 Gy and grown for additional four days. Irradiation by itself decreased DU145 cell growth by 30.6% ($p<0.0001$). However, pretreatment with $1,25(OH)_2D_3$, sodium valproate or with a combination of both drugs reduced PCa cell growth by 46.4%, 83.0% and 87.9%, respectively ($p<0.0001$). Cell-cycle analysis showed that the cell growth-inhibiting effect of these treatments was a result of increased apoptosis and altered cell-cycle distribution.

Irradiation induced apoptosis and caused accumulation of DU145 cells in the S-phase and to a lesser extent in the

G2/M-phase of the cell-cycle in both untreated and pretreated cells. These changes were found to be maximal in the cells pretreated with sodium valproate alone. However, irradiation after combined pretreatment with $1,25(OH)_2D_3$ and sodium valproate had the greatest effect in suppressing PCa cell growth.

In conclusion, the results support our hypothesis that a combination of $1,25(OH)_2D_3$ and sodium valproate is highly efficient in potentiating the anticancer activity of ionizing radiation. As such, we believe that this combined pretreatment may provide the basis for the clinical application of radiotherapy for the treatment of hormone-refractory prostate cancer.

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EFFECT OF ANTI-INFLAMMATORY (*IL-4, IL-10*) CYTOKINE GENES IN RELATION TO RISK OF CERVIX CARCINOMA

Mohammad Shekari[1], Ranbir Chander Sobti[1], Dor Mohammad Kordi Tamandani[1] and Vanita Suri[2]

[1]Department of Biotechnology, Panjab University, Chandigarh;
[2]Department of Obstetrics and Gynecology, Post Graduate Institute of Medical Education and Research, Chandigarh, India;
[3]Research Laboratory, Faculty of Medicine, Hormozgan University of Medical Science, Iran- Bandar-Abbas.

Objectives: Cervical cancer is rated the second most common malignant tumour globally and is an etiologically linked to human papillomavirus (HPV) infection. Interleukin-4 (IL-4) and IL-10 are cytokines with anti-inflammatory properties. The purpose of this study was to determine the relationship of different alleles of *IL-4* and *IL-10* genes as passive smokers and use of oral contraceptives to risk of cervical cancer. *Materials and Methods:* We investigated the association of cervical cancer with two anti-inflammatory (*IL-4, IL-10*) cytokine genes in a case-control study. The study sample comprised 200 cases of cervical cancer and an equal number of matched controls by variables number of tandem repeat (VNTR) and RFLP analysis. *Results:* In this study the *Rp1/Rp2* genotype of *IL-4* gave a borderline increased risk of developing cervical cancer (OR-1.3, 95%CI-0.45-3.64, p=0.8). In the case of passive smokers, we also found a marginal increased risk of cervical cancer with *AC* and combined *AC+CC* genotypes (OR-1.7, 95%CI-0.90-3.34, p=0.1, and OR-1.7, 95%CI-0.90-3.17, p=0.1, respectively). However, non-significant association was observed between the use of oral contraceptives and risk of cervical cancer with these two anti-inflammatory cytokines with different genotypes. *Conclusion:* The present study suggested an increased risk for developing cervical cancer in North Indian female passive smokers having *IL-4 Rp1/Rp2* and *IL-10* (AC) genotypes.

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HERBAL TRADITIONAL SELLER'S PRESCRIPTIONS FOR CANCER

Mahdieh Shojaa, Leila M. Jouybari and Akram Sanagoo

Golestan University of Medical Sciences, Gorgan, Iran

Introduction: Medicinal plants have been used for the treatment of disease in many countries. Traditional medicine has a long history in Iran. Medicinal plants are used to treat some of cancer symptoms, such as pain, which is common with all kinds of cancers. The aim of this study was to explore the medicinal plants prescribed by the traditional herbal sellers in North of Iran, Gorgan. *Materials and Methods:* The study was undertaken in Golestan province in the North of Iran. We conducted open interviews with all of traditional sellers of medicinal plants in Gorgan. All of the interviews were tape recorded. The data were coded and categorized as it is usual in qualitative methods. *Results:* The current survey revealed that thirteen medicinal plants and also some of the vegetables and fruits such as carrot, tomato and garlic are prescribed by the traditional sellers for the cancer remedy. Rosemary, Borage, Salix aegyptiaca are well-known plants for pain remedy of cancer. Milk Thistle, Common Corncokle, Fumitory, Milfoil, Quercus, and Rubra also are recommended for a variety of cancers, such as skin and stomach cancer. *Conclusion:* The data indicated that the use of herbal remedies is very common. Their use seems to be cultural, rather than attributable to decreased access to health care. Most herbs used pose no threat to health. In some cases, remedies may be blended with traditional medical treatments to ensure better patient compliance.

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FURTHER EXPERIMENTAL EVIDENCE OF THE NOVEL RHENIUM AND PLATINUM ANTITUMOR SYSTEM

Natalia Shtemenko[1], Philippe Collery[2] and Alexander Shtemenko[3]

[1]Dnipropetrovs'k National University, Dnipropetrovs'k;
[3]Ukrainian State Chemical Technology University, Dnipropetrovs'k, Ukraine;
[2]Service de Cancérologie, Polyclinique Maymard, Bastia, France

In our previous publications the antitumor properties of dichlorotetra-μ-isobutyratodirhenium(III) (Re1) and dichlorotetra-μ-adamantylcarboxylatedirhenium(III) (Re2) alone and together with cisplatin were shown in the model of

rat-specific Guerin'k carcinoma (T8). Especially good results were obtained when cisplatin was used together with rhenium substances introduced according to the scheme of antioxidant therapy via a new Re-Pt antitomor system. Data on applications of heavy metal compounds in medicine are very significant and a dirhenium *cis*-dicarboxylate compound with propionate ligands was found to be active against some types of cancer in animal models. We found the nature of the ligands situated around the cluster fragments played a role in the phenomena. Here, the data about structure and antitumor properties of *cis*-$[Re_2(GABA)_2Cl_5(H_2O)]2H_2O$ (Re3) alone and in the Re-Pt system was shown and the possible impact of the size and nature of the acid radicals in the molecule of the rhenium cluster to that properties is discussed.

It was shown that Re3 had its own anticancer properties in this model (stronger than that of Re1 and Re2), but enhanced cisplatin action on tumor growth less effectively than these substances. Application of Re3 as a biochemical modulator of cisplatin action was especially successful under application of liposomal forms of Re3 and in the majority of experiments, led to disappearance of tumor cells, and to an increase of quantities of normal RBC forms, their stability and Hb level in blood of tumor-bearing animals. Interaction of liposomal forms of Re3 with red blood cells in experiments *in vitro* showed its cell-stabilizing properties. In the models of tumor growth and hemolytic anemia *in vivo*, liposomal forms had better therapeutic effect in comparison with their solutions.

The process of formation of liposomes of Re3 was investigated by the method of electronic absorption spectra and mechanism of the interaction with lipids is proposed. Encapsulation of a cluster rhenium compounds to lipid coating may have an activation significance for the quadruple Re-Re bond. Antitumor properties of the rhenium dicarboxylates with *cis*-carboxylate groups around cluster rhenium fragment may be explained first of all by the antiradical properties of the quadruple metal-to-metal bond between the two rhenium atoms. The binuclear cluster Re_2^{6+}-fragment is a part of these compounds and includes multiple rhenium – rhenium bonds with δ-component, which plays the role of free radical "scavenger" by virtue of minor energy δ-δ* cleavage. The role of the ligands may be revealed by the rate of interactions with phosphate groups of lipid membranes and other molecules of living cells.

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BIOMOLECULAR CHARACTERIZATION OF GLIOBLASTOMA MULTIFORME AND PERITUMORAL TISSUE

Gigliola Sica

Institute of Histology and Embryology, Faculty of Medicine, Catholic University of the Sacred Heart, Rome, Italy

In recent years, a new level of understanding into the biology of glioblastoma multiforme (GBM) has been reached. Alterations of several tumor suppressor genes and oncogenes have been reported to be critical to the initial steps of neoplastic transformation. The relevance of some growth factor pathways to the development and progression of GBM has been recognized and significant efforts have been made to clarify the mechanisms driving angiogenesis which represents a key event in tumor growth. A possible relationship between GBM stem cells and neural stem cells persisting in the neurogenic zones of the adult brain has been hypothesized. On the contrary, a research delay occurred concerning the biomolecular characterization of peritumoral tissue which may have relevant clinical implications due to the peculiar growth pattern of GBM.

We previously reported the presence of activated MAP kinases in tumor (1st area) and peritumoral tissue of GBM. In particular, we found that phosphorylated ERK1/2 is expressed in tissue surrounding GBM at a distance from < 1 cm to 3.5 cm from the tumor margin. No statistically significant difference existed in the expression between the 1st area and the surrounding tissue. Phosphorylated ERK1/2 immunoreactivity was independent of the presence of neoplastic elements and present not only in activated astrocytes but in apparently normal glial cells also. Phosphorylated (p) JNK followed the same expression pathway. In addition, we demonstrated that the stem cell marker nestin is present in peritumoral tissue even if in a very low cell percentage including neoplastic cells, activated astrocytes and again apparently normal glial cells. Finally, we reported that the ratios pJNK/nestin and (pJNK/total JNK)/nestin in the area located at a distance <1 cm from the tumor margin have a prognostic significance in GBM patients. Nestin was also expressed in the vessel endothelium both in GBM and in peritumoral tissue.

Since it is believed to be a marker for endothelial cells in active proliferation, we hypothesized that a foundation of neoangiogenesis occurred in the tissue surrounding GBM. For this reason, we studied the expression of nestin in association with CD105 (endoglin) in tissue localized at a distance <1 cm (2nd area) and between 1 cm and 3.5 cm from the tumor border (3rd area). CD105 is a proliferation-associated and hypoxia-inducible transmembrane glycoprotein abundantly expressed in proliferating endothelial cells involved in tumoral neoangiogenesis. To quantify the degree of neoangiogenesis, the most vascularized areas in the tissue (hot-spots) were localized and each nestin- or CD105-positive endothelial cell or group of endothelial cells was counted as an individual vessel at ×200 magnification. The mean value of the vessel count in five fields was considered the final value of the microvessel density (MVD). CD105 was exclusively expressed in endothelial cells. CD105-MVD was higher in GBM than in

the peritumoral tissue, no statistically significant difference existing between the 2nd and the 3rd area. A similar trend was observed for nestin-MVD in peritumoral areas. A positive correlation was found for both nestin and CD105-MVD in the 2nd and 3rd areas. A correlation between survival and CD105-MVD was found in the 3rd area. In fact, the median survival time was longer for patients with CD105-MVD values <8.0 compared with patients with higher values. The survival difference was present in the first period of follow-up.

Our results suggest that in the peritumoral tissue a transformation occurs which is probably induced by growth factors produced by the tumor mass and that this process is supported by an activation of vascularization.

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DEVELOPING APTAMERS AS EFFECTIVE ANTICANCER AGENTS OR RECOGNITION UNITS IN BIOSENSOR TECHNOLOGIES

S.C. Simmons[1], M.N. Velasco-Garcia[1], P.E.C. Brenchley[2], E. McKenzie[3] and S. Missailidis[1]

[1]Department of Chemistry and Analytical Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA; [2]Renal Research Laboratories, Manchester Institute of Nephrology and Transplantation; [3]Faculty of Life Sciences, Michael Smith Building, Oxford Road, University of Manchester, M13 9PT, UK

Background: Aptamers are lengths of single or double stranded nucleotides, typically between 22 and 100 bases long, which can be generated to recognise specific small molecules, peptides, proteins, or even cells and tissues. They have shown great potential as diagnostic and therapeutic agents in anticancer treatment, due to their high specificity and affinity to their target molecules. They have also recently found a niche in the field of biosensing due to their advantages over antibodies; being simpler to synthesise, modify and image, as well as their reported superior affinities and ease of use. *Methods:* Single stranded DNA aptamers were selected using a modified SELEX protocol to an enzymatic tumour marker of commercial interest, and were eluted based on their affinity for the target. Using techniques such as ELISA, immunofluorescence and immunohistochemistry, aptamers were shown to specifically recognise their target *in vitro*, whilst affinity constants were measured using fluorescence quenching and quartz crystal microbalance techniques. Functional assays were employed to demonstrate enzyme inhibition and inhibition of cell motility and tissue remodelling, using cell and tissue cultures to demonstrate the aptamers' antagonistic properties prior to the introduction of any modification. Introduction of the aptamers onto screen-printed carbon electrodes offers a further practical application for easy diagnosis at health care point. *Results and Conclusion:* Sandwich ELISA's showed the initial recognition of the aptamers for the target and our studies demonstrated that aptamers did not bind at the same site as a polyclonal antibody raised for the target. Immunohistochemistry and immunofluorescence studies showed a specific recognition of the target by the aptamers, and compared favourably with the polyclonal antibody. Affinity constants ranged from 7×10^7 to 8×10^7, measured by the fluorescence quenching experiments and quartz crystal microbalance. Once applied to the screen-printed carbon electrodes, these results will form the basis for a facilitated practical approach in the diagnosis of cancer.
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CHRONOMICS OF CIRCULATING PLASMA LIPID PEROXIDES, ANTIOXIDANT ENZYMES AND OTHER RELATED MOLECULES IN CERVICAL CANCER PATIENTS

R.K. Singh, R. Singh, V.K. Singh, S. Singh, S. Mehrotra, A.K. Tripathi, U. Singh, O. Schwartzkopff, G. Cornelissen and F. Halberg

Departments of Biochemistry, and Obstetrics and Gynecology, CSM Medical University and Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA

Background: Chronomics (an outgrowth of chronobiology, the study of diversity in time), is the inferential statistical mapping, "imaging'' of time structures in variables in and around us, consisting of rhythms, chaos and trends *i.e.*, of the chronome. The chronome (from chronos, time, and nomos, rule; time structure) of lipid peroxidation and antioxidant defense mechanisms may relate to prevention and curative chronochemotherapeutic efficacy and management. *Patients and Methods:* Thirty five newly diagnosed women with cervical cancer, 30-60 years of age, and 30 age-matched clinically healthy women were synchronized for 1 week with diurnal activity from about 06:00 to about 22:00 and nocturnal rest. Breakfast was around 08:30, lunch around 13:30 and dinner around 20:30. Drugs known to affect the free-radical system were not taken. Blood samples were collected at 6-h intervals for 24 h under standardized conditions. Plasma malondialdehyde (MDA), total lipid hydroperoxide (LOOH), protein carbonyl (PC), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) activities, and serum ascorbate, urate and high-density lipoprotein cholesterol (HDL-C) concentrations and urinary

MDA and Melatonin were also determined. *Results:* A marked circadian variation was demonstrated for each variable in each group by population-mean cosinor ($p<0.01$). In addition to anticipated differences in overall mean value (MESOR), patients differed from healthy volunteers also in terms of their circadian pattern. Conclusion: Mapping the broader time structure (Chronome) with age and multifrequency rhythm characteristics of antioxidants and pro-oxidants is needed for exploring their putative chemotherapeutic role as markers in cancer chronoprevention and management of cervical cancer.

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OXIDATIVE STRESS AND ANTIOXIDANT DEFENCE SYSTEM IN CHRONIC MYELOID LEUKEMIA

Anil K. Tripathi, Rizwan Ahmad, Payal Tripathi, Raj K. Singh and Vinod K. Singh

Departments of [1]Biochemistry and [2]Hemato-Oncology Unit, Department of Medicine, C.S.M. Medical University, Lucknow, India

Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder with a characteristic genetic rearrangement, the Philadelphia chromosome. Oxidative stress, a pervasive condition of increased amount of free radicals is now recognized to be prominent feature of various diseases including leukemias and their progression. The relationship between the levels of well known oxidative stress markers and antioxidants status reflect better health indices and postures. The present study was planned to review the role of oxidative stress and antioxidant defense system in patho-biology of CML. Oxidative stress was assessed in terms of malondialdehyde (MDA), total lipid hydroperoxide (LOOH) and protein carbonyl (PC) content whereas antioxidant status was evaluated in term of reduced glutathione (GSH) and total thiol (T-SH) and total antioxidant status (TAS) levels in plasma of CML patients. Melatonin (MEL) level was measured in urine. *Patients and Methods:* The present study included 82 (male: female; 1.8:1) CML patients and 70 (male: female; 1.5:1) age-sex matched healthy volunteers. Out of 82 CML patients, 66 were in chronic phase (CML-CP) and 16 in accelerated phase (CML-AP). The median age of CML patients was 35 years and that of healthy participants 34 years. Oxidative stress and antioxidant defense system markers in plasma were evaluated by spectro-photometric procedures whereas MEL level was determined in terms of 6-sulphatoxymelatonin excreted in urine by ELISA kit (IBL-Hamburg). *Results:* There was a significant increase ($p<0.05$) in plasma MDA, LOOH and PC levels in CML patients as compared to healthy subjects. Our results also showed that plasma MDA, LOOH and PC levels were markedly elevated ($p<0.05$) in both CML-CP and CML-AP as compared to healthy volunteers. Antioxidant defense system which was measured in term of reduced GSH, T-SH, TAS and MEL was found to be significantly decreased ($p<0.05$) in CML patients and its phases (CML-CP, CML-AP) as compared to healthy participants. During the follow-up of total 66 CML-CP patients for 12 months, 15 patients of CML-CP progressed to the accelerated phase whereas 51 patients remain in CML-CP phase. The mean plasma levels of MDA, LOOH and PC in patients with CML-CP who progressed to CML-AP were found to be higher than in patients with CML-CP who did not progress to the accelerated phase. An elevation in the plasma levels of MDA, LOOH, and PC was observed in CML-CP patients who progressed to CML-AP. The antioxidant defense system in patients with CML-CP who advanced to CML-AP was found to be decreased than in patients with CML-CP who did not progress to the accelerated phase. The antioxidant defense profiles remained decreased in those CML-CP patients who progressed to CML-AP. *Conclusion:* It could be implicated that plasma MDA, LOOH and PC levels may reveal the magnitude of oxidative stress in CML patients whereas reduced GSH, T-SH, TAS and MEL explain the antioxidant defense system against oxidative stress. All these parameters for oxidative stress and antioxidant defense mechanism may precisely reflect the proliferative signal transduction, disease phenotype and its subsequent disease progression. Plasma MDA, LOOH and PC may serve as indices for oxidative stress and disease progression in patients with chronic myeloid leukemia whereas antioxidant defense system plays an important role in nullifying the oxidative stress.

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ROLE OF PROSTATE STEM CELLS IN DEVELOPMENT AND CANCER

Fred Sinowatz, Rebecca Kenngott, David Lincoln and Anwar G. Al-Banaw

Institute of Histology and Embryology, Department of Veterinary Sciences, University of Munich, Munich, Germany

The normal prostate gland displays a high degree of cellular organization. The prostatic epithelium consists of basal cell, now regarded as stem cells of normal prostate growth and fully differentiated secretory cells. The importance of the stem cells varies at different stages of life and prostate development. Prostate development in the foetus occurs when small epithelial cell buds intrude into the stroma and eventually form a complex branched ductular structure. A

hypothesis about the initiation of benign prostatic tumours (BPH), and possibly also about the beginning of neoplastic transformation, suggests that glandular budding may be reactivated in the adult prostate (Schalken and van Leenders, 2003).

Prostate cancer is the most frequently diagnosed cancer in men. Despite advances in the detection of early prostate cancer there is little effective therapy for patients with locally advanced and/or metastatic disease. The majority of patients with advanced prostate cancer respond initially to androgen ablation therapy, however most go on to develop androgen independent tumours that are finally fatal. A similar response is seen to chemotherapy and radiotherapy. As a result, metastatic prostate cancer remains an incurable disease by current treatment strategies.

Recent reports of cancer stem cells have prompted questions regarding the involvement of normal stem/progenitor cells in prostatic cancer. Although still controversial, the cancer stem cell may be a target in the treatment of prostate cancer and a thorough understanding of its biology, particularly of how the cancer stem cells differs from normal stem cells, might allow it to be targeted selectively and eliminated. Recent work on immuno-phenotyping of prostate cancer support the hypothesis that prostrate cancer arises from malignant transformation of intermediate stem cells. In this lecture I will review the molecular mechanism of prostate epithelial cell differentiation during development and cancer. It becomes obvious that the pattern of transcription factor expression controls epithelial cell determination, where the cell is assigned a developmental fate and subsequently cell differentiation, and where the assigned cell now emerges with its own unique character.

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BACKGROUND, REASONS AND BENEFITS USING THE VIENNA PROTOCOL FOR THE TREATMENT OF PAINFUL BONE RECURRENCES WITH ^{153}SAMARIUM-EDTMP

H. Sinzinger[1,2], S. Granegger[2], K. Weiss[3] and J. Hiltunen[2]

[1]Institute for Diagnosis and Treatment of Lipid Disorders and Atherosclerosis (ATHOS), Vienna;
[2]Department of Nuclear Medicine, Medical University of Vienna;
[3]Department of Radiology and Nuclear Medicine, Hospital Wr. Neustadt, Austria

In the 1980s ^{153}Samarium-EDTMP *i.v.* was introduced into pain palliation of late stage cancer. It fastly became evident that the strategy "the earlier the better" is preferable. Based upon the finding that a dose of 1 mCi/kg is not more beneficial as compared to 0.5 mCi/kg concerning bone pain palliation but significantly more affects the bone marrow, in 1996 we described the Vienna protocol for the first time based on repeated treatments on a given schedule (Europ J Nucl Med *27*: 86, 2000). Contraindication for the treatment is a platelet count $<100\times10^3$/μl, a white blood cell count $<100\times10^3$/μl, a red blood cell count $<3\times10^6$/μl, haematocrit <30% and haemoglobin <12 g/l Hg. However, if an abnormally low blood cell count is due to bone marrow suppression by tumor cell infiltration, a beneficial response and even an increase in peripheral blood cell count after therapy has been seen. Platelets are most affected by ^{153}Sm-EDTMP treatment followed by white and red blood cells. Usually, within 3 months, the peripheral blood cell count almost completely returns to the pre-treatment values.

Therapy is performed 5 times at 3-month intervals, followed by 6-, 9- and 12-month intervals with 5 treatments each. The respective treatment intervals are shortened in case of proven disease progression (scintigraphy, MRI). Blood cell count is performed 3 and 6 weeks after therapy as well as immediately before the next one scheduled. Treatment is indicated when more than 1 bone lesion and/or bone pain exists. Bone scintigraphy is performed on the day after therapeutic application. Repeated application clearly shows benefits beyond pain palliation such as tumor marker decrease and lesion regression monitored and proven by various imaging techniques (scintigraphy, MR). PSA after a few weeks may show a temporary increase, while in most of the patients (71%) it decreased after 3 months. To date, however, there is no individual predictor of response available. While the interindividual ^{153}Sm-EDTMP uptake greatly varies, the intraindividual one is rather stable. Concomitant application of biphosphonates does not significantly affect the uptake. Bone uptake does not correlate with treatment response. A significant improvement in quality of life, analgesics consumption, pain score, Karnovsky score and WHO questionnaire has been documented.

Variable response towards treatment indicates that early lesions are more prone to respond as compared to the ones appearing later during treatment. Even osteoclastic lesions respond as far as they show up positively on bone scintigraphy. Pretherapetuic dosimetry makes no sense as at short-term intervals there are signs of stunning. There is some reason to believe that concomitant statin treatment improves benefit as does a higher red blood cell count and higher haemoglobin. Preliminary concomitant chemo-therapeutic and radiotherapeutic treatment data suggest that this design might even induce better results. Although, prospective large studies are lacking so far to define and improve potential therapeutic benefits of this promising approach, preliminary data suggest ^{153}Sm-EDTMP to be a widely underused therapeutic option.

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USING GENETIC MARKERS TO DETERMINE PROGNOSIS OF UVEAL MELANOMA

Karen Sisley

University of Sheffield, Academic unit of Ophthalmology and Orthoptics, K-Floor School of Medicine and Biomedical Sciences, Beech Hill Road, Sheffield, S10RX, UK

Uveal melanomas are ocular tumours affecting the iris, ciliary body and choroid, and are distinctly different from cutaneous melanomas. Their highly aggressive nature and lack of effective treatment for secondary disease, makes understanding their development and genetic background a high priority. Over twenty years ago karyotypic analysis of a few isolated cases, initially reported the consistent involvement of chromosomes 1, 3, 6 and 8. The presence of these chromosome changes in isolation, or sometimes in association, was shown early to correlate with poor prognosis; and perversely also for one chromosome abnormality with a good prognosis. Now twenty years on the focus of research still centres on these changes, but the approach to studying them has changed focus and adapted to include molecular cytogenetics and array based methodologies. By combining other methods and building on these initial studies the use of these genetic markers is providing a highly reliable indictor of prognosis, but is 100% accuracy ever likely to be obtainable?.

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PROGNOSTIC FACTORS IN PATIENTS WITH LOCALLY ADVANCED (UNRESECTABLE) OR METASTATIC PANCREATIC ADENOCARCINOMA: A RETROSPECTIVE ANALYSIS

N. Papadoniou, C. Kosmas, K. Gennatas A. Polyzos, D. Mouratidou, E. Skopelitis, A. Ntokou, J. Sarantonis, M. Tzivras, S. Sougioultzis and N. Tsavaris

HELGO (Hellenic Group of Oncology), Athens, Greece

Background: Most patients with pancreatic adenocarcinoma are diagnosed with locally advanced (unresectable) or metastatic disease. The aim of this study was to investigate possible prognostic factors of survival in such patients. *Patients and Methods:* Two hundred and fifteen patients were studied retrospectively. Twenty-four potential prognostic variables (demographics, clinical parameters, biochemical markers, treatment modality) were examined. *Results:* Mean survival was 29.0 weeks. 21.9% survived more than 36 weeks. On multivariate analysis, 10 factors had an independent effect on survival: tumour localisation, metastasis, performance status, jaundice, weight loss, C reactive protein, CEA, CA 19-9, palliative surgery and chemotherapy. Patients managed only with palliative care had a hazard ratio of 8.94 versus those offered a combination of palliative surgery and chemotherapy. *Conclusion:* Chemotherapy and palliative surgery are associated with increased survival, and should be offered to all eligible patients.

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ASPECTS OF THE ASSOCIATION BETWEEN LEISHMANIASIS AND MALIGNANT DISORDERS

P. Kopterides, E.G. Mourtzoukou, E. Skopelitis, N. Tsavaris and M.E. Falagas

Alfa Institute of Biomedical Sciences (AIBS), Department of Pathophysiology, Oncology Unit, Laiko General Hospital, University of Athens, School of Medicine, Athens, Greece

The aim of this review is to summarise the occurrence of leishmaniasis as an opportunistic infection associated with malignant disorders and to present the available literature potentially linking this infection with the development of cancerous lesions. *Materials and Methods:* We searched electronic databases and evaluated 37 studies involving 44 patients. *Results:* Four different types of association between leishmaniasis and cancer were established: leishmaniasis mimicking a malignant disorder, such as lymphoma; leishmaniasis arising as a difficult to diagnose and treat infection among patients receiving chemotherapy for various malignant disorders; simultaneous diagnosis of leishmaniasis and a neoplastic disorder in the same tissue samples of immunocompromised patients; and direct involvement of Leishmania spp. in the pathogenesis/occurrence of malignant lesions, especially of the skin and mucous membranes. *Conclusion:* Leishmaniasis can directly or indirectly affect the presentation, diagnosis and course of various malignant disorders and it should be considered in the differential diagnosis of malignancies in geographic areas where it is endemic and/or in patients with travel history to these areas.

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72-GENE SIGNATURE PREDICTS RECURRENCE IN LUNG CANCER – RESULTS FROM THE EUROPEAN MICROARRAY CONSORTIUM AMSTERDAM-BIALYSTOK-GDANSK-HEIDELBERG

Marcin Skrzypski

Department of Oncology and Radiotherapy, Medical University of Gdansk, Gdansk, Poland

Background: Current staging methods are imprecise for predicting prognosis of early stage non-small cell lung

cancer (NSCLC). We aimed to develop a gene expression profile for stage I and II NSCLC, allowing identification of patients with a high risk of disease recurrence within 2-3 years after initial diagnosis. *Materials and Methods:* We used whole-genome gene expression microarrays to analyze frozen tumor samples from 172 NSCLC patients (pT1-2, N0-1, M0) from 5 European institutions, who had undergone complete pulmonary resection. Median follow-up was 89 months (1.2-389) and 64 patients developed a recurrence. A random two-thirds of the samples were assigned as the training cohort with the remaining samples set aside for independent validation. Cox proportional hazard models were used to evaluate the association between expression levels of individual genes and patient recurrence-free survival. A nearest mean analysis was used to develop a gene-expression classifier for disease recurrence. *Results:* We have developed a 72 gene expression prognostic NSCLC classifier. Based on the classifier score, patients were classified as either high or low risk of disease recurrence. Patients classified as low-risk showed a significantly better recurrence-free survival in both the training set ($p<0.001$; n=103) and in the independent validation set ($p<0.01$; n=69). Genes in our prognostic signature were strongly enriched for genes associated with immune response. *Conclusion:* Our 72-gene signature is closely associated with recurrence-free and overall survival in early-stage NSCLC patients and may become a tool for patient selection for adjuvant therapy.

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GENE EXPRESSION PROFILING IN NON-SMALL CELL LUNG CANCER CELLS ACCORDING TO SMOKING STATUS

M. Skrzypski[1], A. Szymanowska[1], R. Rosell[2], M. Taron[2], T. Muley[3], H. Dienemann[3], W. Rzyman[1], R. Rzepko[2], M. Jarzab[4], E. Jassem[1] and J. Jassem[1]

[1]Medical University, Gdansk;
[4]Institute of Oncology, Gliwice, Poland;
[2]Catalan Institute of Oncology, Barcelona, Spain;
[3]University, Heidelberg, Germany

Background: Epidemiological and experimental studies confirm that cigarette smoking is the principal causal agent of lung cancer. It is estimated that about 2-5% of all lung cancer patients have never smoked. Knowledge on molecular features of lung cancers not related to smoking might lead to better understanding of their carcinogenesis and allow development of new therapeutic targets. The aim of the study was to assess the feasibility of gene expression profiling to delineate the surgically treated non-small cell lung cancer (NSCLC) patients according to cigarette smoking status. *Materials and Methods:* The study group included 25 non-smoking NSCLC patients and 45 smokers who underwent pulmonary resection with curative intent. Analysis of expression of 27 genes (including genes associated with smoking, kinases, hormonal receptors, growth factor receptors, transcription factors, genes indirectly involved in HPV infection pathways and other) was performed using mRNA derived from tumour tissue for the quantitative analysis of selected genes by RT-PCR with the use of microfluid cards (MFC). Analysis of RT-PCR was performed with relative quantification $2^{-\Delta\Delta Ct}$. Subunit 18S, POLR2A and ESD were used as normalization genes. *Results:* In univariate analysis, three genes were particularly overexpressed in tumors of non-smokers: RRAD ($p=0.0002$), TGF-beta receptor-2 (TGFBR2; $p=0.002$) and progesterone receptor (PgR; $p=0.007$). TGF-beta receptor-3 (TGFBR3; $p=0.02$), SOX9 ($p=0.02$) and androgen receptor (AR; $p=0.03$) were also significantly overexpressed in these tumors. After correction for the impact of sex, histopathology, stage of disease, and multiple comparisons, RRAD and TGFBR2 were independently correlated with smoking history. *Conclusion:* NSCLC in non-smokers is characterized by a specific gene expression signature. Overexpression of TGFBR2 provides the basis for developing new targeted therapies.

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CHARACTERIZATION OF TUMOR-SPECIFIC T-CELLS RESPONDING TO ANTITUMOR MIMOTOPE VACCINES

Jill E. Slansky, Kimberly R. Jordan and Charles B. Kemmler

University of Colorado Denver, Department of Immunology, 1400 Jackson Street K511, Denver, CO 80206, USA

Multiple approaches are being explored to activate T-cells that specifically recognize and kill tumor cells. We are using mimotopes, peptides that mimic tumor epitopes, to activate T-cell-mediated antitumor immunity. Our ultimate goal is to combine these mimotopes with effective adjuvants resulting in potent antitumor vaccines.

Using a T-cell clone specific for the CT26 transplantable tumor, we showed that increasing the affinity of the T-cell receptor (TCR) for the mimotope/MHC complex can augment antitumor immunity. However, not all mimotopes generate effective antitumor immunity. We have shown that increasing the affinity of the interaction too far also ablates the antitumor response. Interestingly, recent experiments showed that relative to vaccination with ineffective mimotopes, vaccination with effective mimotopes generates T-cells with a focused repertoire encoding TCR genes that

are more similar in sequence to the T-cell clone used to identify the mimotopes.

One mechanism that may contribute to improved antitumor immunity is that the T-cells responding to effective mimotopes also produce more IFN-gamma (IFNγ) in response to the tumor antigen. These results were consistent in both the spleen after vaccination and in the developing tumor as determined by intracellular cytokine assays. In IFNγ-deficient mice, we showed that IFNγ is not required for an effective *primary* response to mimotopes as determined in a tumor protection assay and tetramer staining of tumor-specific T-cells. However, IFNγ may be required for an effective *memory* response. Specifically, mimotopes protect wild type mice, but not IFNγ-deficient mice, from tumor challenge if challenged 3 weeks after the first vaccine. Thus, our future experiments are to determine the predictable sequence of mimotopes that generate a focused repertoire of responding CTL that produce IFNγ upon secondary challenge.

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MOLECULAR MECHANISMS RESPONSIBLE FOR THE ANTICANCER ACTIVITY OF EDIBLE AND MEDICINAL MUSHROOMS

Daniel Sliva

Cancer Research Laboratory, Methodist Research Institute, Indianapolis, IN;
Department of Medicine, and Indiana University Simon Cancer Center, School of Medicine, Indiana University, Indianapolis, IN, USA

Cancer metastasis is among the major reasons for a high mortality of cancer patients. Therefore, inhibition of growth, invasive behavior and cancer-cell mediated angiogenesis will lead to the suppression of cancer metastasis. Although some of the anticancer drugs were originally identified and developed from a variety of natural products, the discovery of novel biologically active compounds is still of vital interest. From the total of estimated 1.5 million Earth's fungi only 100,000 have been described. Some of these fungi gained significant recognition as the part of nutrition or as medicinal mushrooms in the traditional Oriental medicine. Polysaccharides, mainly beta-glucans, are usually associated with the anticancer activities of mushrooms through the stimulation of immune system. Moreover, the biologically active compounds in mushrooms can directly modulate aberrantly activated signaling pathways responsible for growth and invasive behavior of cancer cells. Here, we compare the activity of edible and medicinal mushrooms and their extracts *Pleurotus ostreautus* (Oyster), *Grifola frondosa* (Maitake, D-Fraction), *Ganoderma lucidum* (Reishi), and *Phellinus linteus* (Meshimakobu, PL-Fraction). Thus, D-Fraction inhibits proliferation and suppresses invasiveness of breast cancer cells. Moreover, PL-Fraction (PLF) inhibits proliferation as well as colony formation through the cell cycle arrest and the up-regulation of p27Kip1 expression. PLF also inhibits invasive behavior of breast cancer cells through the suppression of secretion of urokinase-plasminogen activator (uPA). PLF inhibits the early event in angiogenesis, capillary morphogenesis of the human aortic endothelial cells (HAEC), through the down-regulation of secretion of vascular endothelial growth factor (VEGF) from cancer cells. These effects are mediated by the inhibition of serine-threonine kinase AKT signaling because PLF suppresses phosphorylation of AKT. In summary, edible as well as medicinal mushrooms contain biologically active compounds with anti-proliferative, anti-invasive and anti-angiogenic activities, suggesting their potential therapeutic effect against invasive cancers. Finally, we have identified the most active fraction responsible for the PLF activity, and this fraction is currently evaluated in animal experiments.

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CANCER ASSOCIATED FIBROBLASTS: FACTOR OF TUMOR PROGRESSION AND SPREADING

Karel Smetana Jr.

Charles University, First Faculty of Medicine, Institute of Anatomy and Second Faculty of Medicine, Center of Cell Therapy and Tissue Repair, Prague, Czech Republic

Malignant tumors are widely spread in humans and form a serious medical, economical and social burden. Aging of the population can be related to the increased incidence of malignancies. Despite progress in cancer therapy, the prognosis of many patients does not seem to be optimistic. Remarkable progress in stem cell research delineated new horizons of possible future improvement of cancer therapy. A paradigm of existence of cancer stem cell has been established in solid tumors and is based on the remarkable parallel between tissue stem cells and population of cancer cells responsible for tumor spreading. Normal tissue stem cells require highly specialized microenvironment that is necessary for the maintenance of their stemness; a disorder of such a microenvironment drives the stem cell population to enter into the process of final differentiation. Experiments based on embryonic stem cell introduction into adult organisms resulting in tumor development are well-known. On the other hand, tumor cells in embryos frequently normalize their function.

These observations indicate the importance of microenvironment in tumor biology. The positive role of tumor stroma in course of vascularisation of tumor bed has already been well described. The sum of evidence that cancer-

associated stromal fibroblasts exert an important role in cancer progression is growing and their participation in cancer stem cell niche formation seems to be highly probable. The differences between the cancer stroma and normal tissue fibroblasts were established in *e.g.* tumors of breast, pancreas, colon, prostatic gland, skin and oral cavity. When co-cultured with cancer-associated fibroblasts, tumor cell lines harden their cancerous phenotype and the inoculation into immunodeprived mice leads to the rapid tumor progression including metastasis. Normal keratinocytes subsequently alter their phenotype to resemble cancer cells when co-cultured with stromal fibroblasts prepared from basal or squamous cell carcinoma. Modern analytical technologies, such as DNA microarrays and proteomic analysis, indicated differences in production of regulatory factors/cytokines that are significant in the biological activity of cancer-associated fibroblasts. The nature of these fibroblasts is not well understood yet, but principally they can originate in local mesenchyme under the control of cancer cells. However, they can also arise from tumor cells undergoing epithelial-mesenchymal transition or in cells formed by fusion of cancer cells with local fibroblasts. Summarizing these data, similarly to embryonic development, mesenchymal-epithelial interaction can play an important role in tumor progression and its management may be a promising future anticancer therapeutic tool.

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NUCLEIC ACID CONFORMATION AND HOTSPOTS FOR DNA METHYLATION

Steven S. Smith

City of Hope, 1500 E. Duarte, CA 91010, USA

Nucleic acid conformation space is extraordinarily vast. The conformations that DNA can adopt are nearly uncountable. Modern living organisms utilize only a fraction of the available conformations, probably because of the constraints placed on DNA by fidelity in genetic inheritance. I will summarize evidence suggesting that DNA methylation is one of the systems that allows higher organisms to incorporate sequences with difficult-to-manage conformations into their genomes. In particular we will discuss evidence suggesting that a non-B DNA structural polymorphism detected in human tumors near the c-Ha-ras VNTR is a self-perpetuating epigenetic mark that manifests itself spontaneously during breast carcinogenesis in a hotspot for DNA methylation. Available evidence suggests that DNA hypermethylation and concurrent DNA hypomethylation in carcinogenesis can be understood as consequences of loss-of-function in the suppression of non-B DNA and unusual DNA structure formation.

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PHOTODAMAGE TO PROTEINS OF DNA REPLICATION AND REPAIR BY TOPOISOMERASE INHIBITORS

Soo In Bae, Ran Zhao and Robert M. Snapka

Department of Radiology, Division of Radiobiology, The Ohio State University, Columbus, Ohio USA

We have found that commonly studied drugs, including topoisomerase II and topoisomerase I inhibitors, cause covalent damage to proliferating cell nuclear antigen (PCNA) and SV40 large T antigen when cells treated with micromolar levels of these drugs are exposed to fluorescent light. PCNA and large T antigen function as circular oligomers, and the subunits are covalently cross-linked by photodamage from these drugs. With some drugs, significant cross-linking of PCNA or large T antigen can be detected after 5-minute exposures of drug-treated cells to laboratory room lighting. Heterologous proteins are photo-cross-linked to the PCNA oligomers at much lower rates. Photodynamic drugs known to localize in cytoplasmic organelles also caused photo-cross-linking of PCNA and large T antigen, suggesting that small amounts of these drugs, or long-lived reactive species generated in the cytoplasm can reach the nucleoplasm. PCNA and large T antigen can serve as very sensitive markers of photodynamic damage in the nucleus. Tests for specific reactive oxygen species suggest that singlet oxygen is involved in PCNA photo-cross-linking. Nuclear photodamage is unlikely to be limited to protein cross-linking or to be limited to these two proteins. Damage to proteins of DNA replication and repair has the potential to disrupt DNA replication as well as DNA damage signaling and processing, resulting in genetic instability. This work was supported by NIH/NCI RO1-CA097107 to RMS and the Ohio State University Comprehensive Cancer Center.

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CLINICAL AND TUMOR BIOLOGICAL ASPECTS OF APOLIPOPROTEIN–D IN OPERABLE BREAST CANCER

Håvard Søiland

Stavanger University Hospital, Stavanger, Norway

Apolipoprotein D (ApoD) is a small glycoprotein of 24 kDa, which belongs to the lipocalin family, unlike other apolipoproteins. ApoD is linked to high-density lipoprotein in plasma. It may also be highly expressed in breast, adrenal and nerve, tissue, where transportation of steroids and lipophilic substances is abundant. The affinity of ApoD to arachidonic acid, progesterone and tamoxifen makes it

relevant in breast cancer. The emerging understanding of the relationship between ApoD and cellular signaling pathways (*e.g.* estrogen receptor-alfa) and also the connections to cellular stress and senescence will be addressed.

Recent studies on qualitative and quantitative immunohistochemical aspects of ApoD determination in breast cancer tissue will be presented. Moreover, recent observations indicate a prognostic value of ApoD in certain subgroups of breast cancer patients. Furthermore, we hypothesize on the possible predictive value of ApoD for effect of adjuvant tamoxifen in a subset of women with operable breast cancer.

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DEMONSTRATION OF THE ACTIVITY OF P-GLYCOPROTEIN BY A FULLY AUTOMATED ETHIDIUM BROMIDE METHOD

Gabriella Spengler[1,2], Miguel Viveiros[1], Ana Martins[1], Liliana Rodrigues[1,2], Marta Martins[1,2], Joseph Molnar[3], Isabel Couto[1,4] and Leonard Amaral[1,2]

[1]Unit of Mycobacteriology and [2]Unidade de Parasitologia e Microbiologia Médicas (UPMM), Instituto de Higiene e Medicina Tropical, and [4]Centro de Recursos Microbiológicos (CREM), Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa (IHMT/UNL), Lisboa, Portugal;
[3]Institute of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Szeged, Hungary

The major reason for the failure of chemotherapy of cancer is the refractory nature of the cell that resulted from prior exposure to the same or another chemotherapeutic agent. The refractory nature of that cell is due to over-expression of the mdr-1 gene that codes for the transporter Pgp-1 that promotes the extrusion of widely dissimilar cytotoxic agents employed in cancer therapy. Agents which inhibit this extrusion render the cancer cell susceptible to the agent to which it had become resistant. Obviously, targeting this transporter is of interest.

The effect of an agent on the Pgp-1 is normally assessed using flow cytometry. This involves the employment of a fluorochrome substrate, such as rhodamine 123, which is extruded by the P-gp-1 transporter and which is increasingly retained if the transporter is inhibited. Although the sensitivity of this method is quite good, it does not lend itself to physiological conditions, it is very time consuming, data is not readily reproduced and hence of limited use for its standardization, and of course, utilizes a very expensive instrument at its core. Furthermore, the method does not lend itself for the evaluation of large numbers of assays to be conducted within a working day.

We developed an automated method that utilized the fluorochrome ethidium bromide (EB) which is considered as a universal substrate of bacterial efflux pumps. Because EB is also recognized and extruded by ABC type transporters that rely on the ATP binding cassette of Gram positive bacteria, and this transporter has similarity to the Pgp-1, we have extended the method for the evaluation of agents that can inhibit the extrusion of EB by mouse lymphoma cells that contain the human mdr-1 gene, and are therefore multi-drug resistant. The data presented shows that phenothiazines, such as thioridazine and its derivatives inhibit the extrusion of EB under physiological conditions. The activity of other unrelated agents such as reserpine, verapamil, SILA compounds, *etc*., has also been evaluated and will be presented.

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SULINDAC SULFIDE: POTENT INTERACTION WITH CYTOSTATICS

Sylwia Flis and Jacek Spławiński

National Medicine Institute, 34 Chelmska Str, 00-725 Warsaw, Poland

Objectives: To compare the effects of sulindac sulfide (SD) with other COX inhibitors on survival of colorectal carcinoma (CRC) cells in various cell lines (Colo 205, SW48, HT 29). *Results:* SD (a metabolite of sulindac) [at conc. of 25 to 150 μM], celecoxib, and SC58125 dose dependently inhibited growth with G_1-phase arrest, while rofecoxib, valdecoxib, and indomethacin were ineffective. The arrest of cell cycle progression was accompanied by increased level of p21 protein, accumulating in G_1-phase, and decreased level of cyclin B1. Cell cycle arrest was accompanied by strong apoptosis (65.7% of apoptotic cells). SD induced increase of transcript of *caspase-3* gene, as well as, reduction of pro caspase 8 and 3 proteins level. In addition, incubation of Colo 205 cells with SD led to decrease of Bid and Bax protein level and PARP protein cleavage. Our results indicated that both mitochondral and death receptor pathways of apoptosis were stimulated. In contrast to celecoxib and SC58125 no cytotoxicity in normal Balb/c 3T3 was observed with SD. Thus, SD appeared as an optimal candidate to study interactions with 5 fluorouracil (5 FU) and oxaliplatin (OXA) in CRC cell lines. SD synergistically with 5 FU and OXA inhibited CRC survival, in contrast to celecoxib, which gave additive or antagonistic (depending on the line studied) effects. Dose reduction effect of SD with studied cytostatics was in the range of 5 to 14 fold, when compared with single agent. Synergistic effect of SD with 5 FU and OXA on growth of CRC cells was paralleled by changes in the cell cycle progression and induction of apoptosis. *Conclusion:* Strong apoptotic signal induced by SD *via* both intrinsic and extrinsic pathways may explain observed inhibitory synergism with 5

FU and OXA on CRC cells and superiority to combination of 5 FU and OXA with celecoxib. Since SD may be formed in intestines from sulindac and is preferentially concentrated in the colon *in vivo*, sulindac may constitute a candidate for the "fourth line" therapy of patients with CRC.

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PULSE-MEDIATED CHEMOTHERAPY OF SOLID TUMORS IN PETS (ELECTROCHEMOTHERAPY): RECENT DEVELOPMENTS OF A NOVEL ANTICANCER THERAPY

Enrico P. Spugnini, Alfonso Baldi and Gennaro Citro

Regina Elena Cancer Institute, Rome, Italy

Electrochemotherapy (ECT) couples the administration of an anticancer agent (usually bleomycin or cisplatin) to the delivery of electric pulses having appropriate waveforms. The application of permeabilizing pulses leads to perturbation of the cell membrane, thus resulting in increased uptake of chemotherapeutic drugs, ultimately leading to cell apoptotic death. Different waveforms have been adopted by investigators: exponentially decaying, square, rectangular or biphasic. In general the number of pulses delivered is set in 8 single pulses applied per cm of tumor area at a voltage of 1300 V/cm (800 V/cm for intraoperative use), with a duration of 100 μs and a frequency of 1 Hz. Treatments are repeated until the whole tumor area is covered. Our group investigated in the past 8 years the feasibility of electrochemotherapy in companion animals affected by different neoplasms. We adopted trains of biphasic pulses delivered as a burst instead of single impulses. The electric waveform was generated by *Chemopulse* equipment. In the first trial, ECT was used to directly attack neoplasms (1). Overall response rate was 80%, with 40% long lasting remissions (in excess of 1 year). On the basis of the preliminary studies, cohorts of dogs and cats affected by melanoma, soft tissue sarcoma, mast cell tumor, squamous cell carcinoma and cutaneous lymphoma were enrolled in phase II studies, obtaining response rates and remissions that positively compared with those reported in the literature using standard protocols (2-10). Electrochemotherapy is a safe and efficacious approach to veterinary soft tissue and cutaneous neoplasms. Its low cost and ease of administration make it a valuable addition to the currently available oncological therapies. Results obtained in companion animals could be instrumental in planning new protocols for humans.

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FEEDBACK REGULATION BETWEEN HOXC GENES AND ESTROGEN/RTK SIGNALING IN MAMMARY GLAND DEVELOPMENT AND CANCER

Alejandra García-Gasca[1], Carola A. Neumann[2], Carlos S. Moreno[3], Ioanna G. Maroulakou[4], Steven A. Rosenzweig[2] and Demetri D. Spyropoulos[5]

[1]Laboratorio de Biología Molecular, CIAD Mazatlán, Mazatlán Sinaloa, México;
[2]Pharmacology, and [5]Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC;
[3]Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA;
[4]Molecular Oncology Research Institute, Tufts-New England Medical Center, Boston, MA, USA

Background: Early menarche and breast development are increasingly common risk factors for breast cancer. *In utero* and neonatal exposure to natural and anthropogenic steroids, such as estrogens, can recapitulate early menarche and aberrant mammary development in animal models. Strikingly however, a proliferative response to estrogens is not observed in mammary epithelium until just before puberty. HoxC6 knockout mouse studies indicate essential roles in postnatal mammary development and suggest mechanisms for the actions of estrogens in aberrant breast development and increased carcinogenic potential. *Objectives:* To determine the molecular basis for the essential roles of HoxC6 and to identify other Hox genes essential in mammary development. *Results:* HoxC6 is expressed in mammary epithelium and stroma and therein differentially repressed by ovarian hormones. This repression can also be modulated *in vivo* by phytoestrogens. Thoracic mammary glands fail to develop post-natally in HoxC6 knockout mice. Epithelial-stromal reconstitution experiments (surgical and genetic) show that epithelial-specific, activated Her2/Neu expression is sufficient to rescue ductal elongation defects in the knockout, but that

stromal HoxC6 expression is required for ductal side-branching. Embryonic and neonatal induction of epithelial-specific HoxC6 expression in transgenic mice leads to precocious terminal end bud formation, suggesting distinct cell-autonomous and non-autonomous functions. In human breast epithelial cell lines, real time gene expression analyses (RT-qPCR) reveal that HoxC6 expression is elevated in well-differentiated cell lines (MCF10A and MCF7) and reduced in more aggressive cell lines (*e.g.* MDA-MB-231) and tumors. Consistent with *in vivo* HoxC6 knockout results, siRNA-mediated HoxC6 knockdown in MCF10A cells results in an inhibition of growth and branching in 3-dimensional cultures. Rescue of growth (but not morphological) defects in knockdown MCF10A cells is achieved to varying extents through myristoylated Akt isoform up-regulation. Estrogen-mediated repression of HoxC6 in MCF7 cells can be suppressed via Akt isoform-specific up-regulation. In addition, estrogen-mediated changes in MCF7 cellular growth and migration are reversed via Akt isoform-specific up-regulation. This Akt isoform-specific activity does not appear to function *via* alterations in ERα (ESR1) subcellular localization. These results will be discussed in relation to wortmannin, tamoxifen and ICI treatments. *In vitro* induction of adipogenesis and/or senescence in fetal fibroblasts (WI-38 human fetal lung & mouse embryonic day E14) are profoundly altered via siRNA-mediated HoxC6 knockdown. Using chromatin immunoprecipitation (ChIP), we have identified several direct targets of HoxC6 repression (IGFBP3, IGFBP7) and activation (CD44, FGFR2). *In vivo* and *in vitro* disruption of HoxC6 expression (either directly by knockdown/out or indirectly *via* estrogen treatment) leads to a disruption of target gene expression. Estrogen-mediated repression and mammary gland defects observed in HoxC6 knockout mice are recapitulated in HoxC5 knockout mice. Of note, these phenotypes are partially rescued in HoxC6 knockout mice bearing a homeobox-defective, truncated HoxC5 allele, suggesting functional redundancy and dosage dependence of phenotype. Collectively, these studies implicate HoxC6 and HoxC5 as essential regulators of postnatal mammary gland development. This regulation is likely to entail growth, survival and differentiation of epithelium and stromal fibroblasts and adipocytes. *Conclusion:* Identification and modulation of key network regulators hold future promise for the prevention, diagnosis and treatment of various disorders. Dysregulation in steroid hormone/growth factor networks have been implicated in cardiovascular diseases, cancers, diabetes and obesity. Hox genes, their targets and their regulation (*e.g. via* histone methylation/acetylation) can be modeled in the context of ESR signaling: a) the "classical" nuclear-initiated signaling; and, b) membrane-initiated (IGF1R/EGFR/GPCR) signaling. One such model involves a feedback loop in which prepubertal exposure to estrogens causes up-regulation of both IGF1 (*via* ESR1) and IGFBPs (*via* HoxC6 repression) and ensuing aberrant mammary epithelial growth associated with elevated carcinogenic potential.

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IDENTIFICATION OF POTENTIAL BIOMARKERS TO DIFFERENTIATE HUMAN CHOLANGIOCARCINOMA AND HEPATOCELLULAR CARCINOMA FROM CELL LINES

Chantragan Srisomsap[1,2], Pantipa Subhasitanont[1], Phannee Sawangareetrakul[1], Daranee Chokchaichamnankit[1], Khajeelak Chiablaem[1] and Jisnuson Svasti[1,2,3]

[1]Laboratory of Biochemistry, Chulabhorn Research Institute, Bangkok;
[2]Chulabhorn Graduate Institute, Bangkok;
[3]Department of Biochemistry and Center for Protein Structure and Function, Faculty of Science, Mahidol University, Bangkok, Thailand

Cholangiocarcinoma (CCA), a malignant tumor derived from bile duct epithelium, occurs with a higher incidence in tropical countries such as Thailand. Distinguishing CCA from hepatocellular carcinoma (HCC) of the liver often requires the use of histochemistry, so molecular markers for diagnosis and prognosis are still required. More in depth analyses using subproteomic approaches were performed to facilitate the identification of potential biomarkers undetected by regular proteomic methods. The two-dimensional protein map of a Thai human bile duct epithelial carcinoma cell line (HuCCA-1) was compared to those of human hepatocellular carcinoma cell lines (HepG2 and HCC-S102). The major proteins were identified by LC/MS/MS.

Cytokeratins CK8 and CK18 were overexpressed in both HuCCA-1 and HCC, while CK7 and CK19 were only expressed in HuCCA-1. Galectin-3 showed high expression in HuCCA-1 by 1-DE immunodetection. Thus, certain proteins, namely CK7, CK19 and galectin-3, may be good markers useful for differential diagnosis of CCA from HCC.

Proteins secreted (the secretome) from cancer cells are potentially useful as biomarkers of the disease. Subproteomes enriched in membrane proteins or in cytosolic proteins from the HuCCA-1 and the HCC-S102 cell lines were prepared. Protein patterns of differential protein expression were determined. Ten membrane proteins were found in HuCCA-1 but not in HCC-S102, including integrin alpha-6 precursor, ezrin, hippocalcin-like protein 1, mitogen-activated protein kinase kinase kinase 2 (MAPK/ERK kinase kinase 2) and calgizzarin. We

identified proteins in the conditioned media of HuCCA-1, HCC-S102, HepG2 and two other hepatocellular carcinoma cell lines (SK-Hep1 and Alexander). The secretomes of CCA and HCC cell lines were analyzed by SDS-PAGE combined with LC/MS/MS. Procathepsin B was found to be highly expressed in SK-Hep1 and HepG2 but slightly expressed in HCC-S102 and Alexander, while heavy chain cathepsin B was slightly expressed in both cell lines. These results suggest analysis of the proteome and secretome are useful for identification of potential biomarkers of human CCA and HCC cell lines.

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DNA MICROARRAYS AS TOOLS FOR CANCER STEM CELL RESEARCH

Martin S. Staege

Department of Paediatrics, Martin-Luther-University Halle-Wittenberg, Halle, Germany

DNA microarrays are powerful tools for the characterisation of gene expression profiles from cancer cells and their normal counterparts. However, for many cancer types, the cell of origin (the mother cell) is still unknown. Examples are tumours of the Ewing tumour family (EFT). Since the first descriptions of EFT in the late 19th century different cell types have been implicated as the EFT mother cells, ranging from endothelial cells to primitive mesenchymal cells. Recently, similarities between DNA microarray data from bone marrow-derived mesenchymal stem cells (bmMSC) and EFT suggest that bmMSC are highly attractive candidates as EFT mother cells. However, not all EFT associated genes are up-regulated in MSC after transgenic expression of EFT-specific EWSR1-ets oncogenes. Likewise, not all EFT-associated genes are down-regulated in EFT after knockdown of these oncogenes. EFT-specific expression of EWSR1-ets independent genes might be a consequence of *in vivo* selection. In addition, the number of so-called secondary alterations in EFT is very high, rendering the possibility likely that other factors significantly modulate the gene expression profile of EFT. Moreover, other cell types, *e.g.* neuroblastoma cells, acquire features of EFT after transgenic expression of EWSR1-ets, suggesting that the effect of EWSR1-ets on gene expression is not absolutely specific for bmMSC. Alternatively, this behaviour may only reflect the susceptibility of bmMSC to EWSR1-ets mediated transformation.

We analyzed the expression profile of EWSR1-ets-independent genes in more detail. Surprisingly, we found a very high similarity between EFT and embryonic stem cells (ESC). Unsupervised clustering indicated that the similarity between EFT and ESC was even higher than the similarity between EFT and bmMSC. In addition, we observed weak expression of ESC-specific markers, *e.g.* the transcription factor NANOG in EFT. ESC-like cells have been detected in adult tissues. In addition, mesenchymal stem cells from different sources are highly heterogeneous and more primitive mesenchymal stem cells with ESC like differentiation capacities have been described. Our data suggest that primitive ESC-like stem cells, *e.g.* the recently described very small embryonic-like stem cells (VSEL) might be interesting candidates as EFT mother cells.

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CHARACTERIZATION OF TUMOUR ASSOCIATED AND TISSUE SPECIFIC GENE EXPRESSION PROFILES

Daniela Max, Grit Weißflog, Christoph Hutter, Jenny Kaftan, Christine Mauz-Körholz, Dieter Körholz and Martin S. Staege

Department of Paediatrics, Martin-Luther-University Halle-Wittenberg, 06097 Halle, Germany

DNA microarray-detected signal intensities of tumour associated genes are characterized by a high variance in the total group of samples (including normal and tumour samples) compared with the variance in normal tissues alone. Therefore, the ratio of the variance in normal tissues and the total variance (Wilks' lambda score, WLS) can be used for the identification of tumour specific genes. These genes are characterized by a low WLS. *Vice versa*, a high WLS characterizes tissue specific genes that are only expressed in a subset of normal samples. We used this approach for the characterization of gene expression profiles of Ewing family tumours (EFT) and Hodgkin's lymphoma (HL). For this end, we combined published microarray data from tumour samples and normal tissues from our own lab and from the GEO data base. In our analysis of EFT we found among the genes with low WLS the complete set of EFT associated genes that we had identified in our previous studies, indicating that the method gives reliable results. Similar results were obtained during analysis of HL samples. One of the genes with lowest WLS in this analysis was interleukin 26 (IL26), despite the fact that IL26 was expressed only in a subset of HL cell lines. Interestingly, IL26 expression was induced in other cells after incubation with culture supernatants from IL26 positive HL cells, suggesting that HL cells secrete soluble factors that induce IL26 in bystander cells. In addition, we identified a set of tissue specific genes (high WLS) that were expressed only in small subsets of normal samples, *e.g.* insulin (expressed only in pancreas) or the Charcot-Leyden crystal protein (expressed in blood and bone marrow). Among these genes we found several testis specific genes (*e.g.* protamines or the glyceraldehyde-3-

phosphate dehydrogenase S), as well as cancer/testis antigens (CTA). These CTA include members of the GAGE (G antigen) family, members of the SPANX (sperm protein associated with the nucleus, X chromosome) family, lactate dehydrogenase C, and the outer dense fiber of sperm tails protein 2. Our results demonstrate that WLS can be used not only for the identification of tumour associated genes but also for the identification of tissue specific genes including cancer/testis antigens.

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PROTEOMICS AND PATHWAY ANALYSIS IDENTIFIES JNK-SIGNALING AS CRITICAL FOR HIGH-LET RADIATION-INDUCED APOPTOSIS IN NON-SMALL LUNG CANCER CELLS

Sara Ståhl[1], Eva Fung[2], Christopher Adams[2], Johan Lengqvist[1], Birgitta Mörk[1], Bo Stenerlöw[3], Rolf Lewensohn[1], Janne Lehtiö[1], Roman Zubarev[2] and Kristina Viktorsson[1]

[1]Department of Oncology/Pathology, Karolinska Biomics Center, Karolinska Institutet, Stockholm; [2]Division of Molecular Biometry, Institute for Cell and Molecular Biology, and [3]Unit of Biomedical Radiation Sciences, Department of Oncology, Radiology and Clinical Immunology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden

Low linear energy transfer (LET) radiation is used on regular basis for cancer therapy. However, an improved efficiency of radiotherapy in non-small-cell lung carcinomas (NSCLC) by using high-LET radiation has been described. To date, little is known about which signaling pathways are responsible for this increased efficiency. Here we have used proteomics and pathway analysis to reveal signaling networks of importance for proapoptotic signaling in response to high-LET radiation.

The NSCLC cell line U-1810, resistant to low-LET but sensitive to high-LET radiation was used as model system. Cells were harvested 4h after irradiation with low and high-LET radiation, respectively. An unsupervised approach was applied using shotgun proteomics where high resolution mass spectrometry coupled to nanoflow-liquid chromatography determined the identity and relative abundance of expressed proteins. A newly developed pathway search engine (PSE) was then employed to determine the pathway status in both low and high-LET irradiated cells. The observed differences in the pathway domain were used to generate hypotheses which were validated *in vitro*.

Based on the 650 proteins quantified in each sample, the JNK-pathway was identified ($p=6\cdot10^{-6}$) as a key event in response to high-LET radiation-induced apoptotic signaling. In addition, the Fas-pathway was activated ($p=3\cdot10^{-5}$) and the p38-pathway was found deactivated ($p=0.001$) compared to untreated cells. Western blot, ELISA and immunofluorescence analyses confirmed that high-LET radiation caused an increase in phosphorylation of JNK. Moreover, pharmacological inhibition of JNK blocked high-LET induced apoptotic signaling. In contrast, neither an activation of p38 nor a role for p38 in high-LET radiation induced apoptotic signaling was found.

We conclude that in contrast to conventional low-LET radiation, high-LET radiation can trigger activation of the JNK-pathway which in turn is critical for induction of apoptosis in these cells. Thus PSE predictions were largely confirmed, and PSE was proven to be a useful hypothesis-generating tool.

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DETECTION OF BIOLOGICAL PROFILE OF CUTANEOUS MALIGNANT MELANOMA: A ROLE FOR CAF-1/P60 PROTEIN AND STEM-CELL MARKER CD133 EXPRESSION?

Stefania Staibano[1,3], Massimo Mascolo[1], Maria Di Benedetto[2], Gennaro Ilardi[1], Maria Luisa Vecchione[1], Gaetano Salvatore[2], Loredana Nugnes[1] and Gaetano De Rosa[1,3]

[1]Department of Biomorphological and Functional Sciences, Pathology Section, [2]Department of Medicine, and [3]Cooperative Melanoma Group, "Federico II" University, School of Medicine, Naples, Italy

Cutaneous malignant melanoma (CMM) is the most aggressive and lethal among skin cancers. To date, no reliable molecular prognostic marker exists for the evaluation of the biological behaviour of this tumor. Recently, a great interest has been devoted to the inter-relationship between chromatin organization, DNA damage processing and the control of the "cell cycle checkpoint machinery". The resetting of the pre-existing chromatin structure during DNA synthesis, DNA replication, and/or DNA repair is driven, in part, to the Chromatin Assembly Factor 1 (CAF-1). CAF-1 is a heterotrimeric protein complex formed of three subunits (p48, p60 and p150). CAF-1 interacts directly with proliferating cell nuclear antigen (PCNA), and the p60 CAF-1 subunit expression has been recently correlated with cell proliferation in cell lines and human tissue, and it has been proposed as a novel sensible proliferation and prognostic marker in some tumours.

We aimed to establish if CAF-1p60 could have a role as a new prognostic indicator in a selected series of cutaneous malignant melanomas (CMM). 80 formalin-fixed, archival paraffin-embedded surgical specimens of CMM were retrieved from the files of the Department of Biomorphological and Functional Sciences, Section of Pathology, University Federico

II of Naples. Immunohistochemistry with anti-CAF-1 p60 was performed on four-micron-thick tissue sections for all cases, and the staining was evaluated semi-quantitatively. Results were compared with clinicopathological features of tumours and with follow-up data of patients. Moreover, we compare the results with the immunostaining pattern of the same cases for the stem–cells CD133 antibody. Recent studies, in fact, have pointed the attention on the possible role of stem-cells derivation in conditioning the malignant progression of some cases of CMM.

We found CAF-1/p60 overexpressed in almost all CMM of our series, with values exceeding from two-to ten fold the level of expression of melanocytes of normal control skin. The statistical analysis of results demonstrated a significant association between the hyperexpression of CAF-1/p60 and the occurrence of node and/or distant metastases in CMM patients ($p<0.01$). On the contrary, only a minority of CMM (9 out 80 cases) showed an appreciable reactivity for the stem-cell marker CD133, without any convincing relationship with the biological behavior of tumors and/or the main classical clinical and pathological prognostic parameters. These results indicate that CAF-1/p60 may have a role as new sensible proliferation and prognostic marker in CMM, whereas the derivation from the stem-cell compartment does not seem useful for the prediction of clinical outcome in CMM patients.

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WHICH ABC-TRANSPORTERS SHOULD WE TARGET IN LEUKEMIA?

Daniel Steinbach

Universitätskinderklinik Ulm, Germany

ABC-transporters are a large family of proteins involved in active transport across biological membranes. Some members of this family cause drug resistance in malignant diseases *via* ATP-dependent drug efflux from malignant cells. This phenomenon was intensively analyzed in leukemia.

ABCB1 (P-g) and ABCG2 (BCRP) were shown to be associated with poor response to chemotherapy in adult acute myeloid leukemia (AML). Both proteins confer resistance to a wide range of chemotherapeutic drugs *in vitro*. Therefore, they represent possible therapeutic targets. In pediatric AML, this is the case for ABCG2 but not for ABCB1. In acute lymphoblastic leukemia (ALL), both proteins appear less relevant with the probable exception of ABCB1 in adult patients.

ABCC3 (MRP3) has a strong prognostic impact in AML and ALL, independent of age group. However, ABCC3 does not cause much drug resistance *in vitro*. Therefore, it remains to be elucidated whether its correlation with poor response to therapy is causative or just an epiphenomenon.

ABCA3 might be an additional cause of drug resistance in AML. It is associated with *in vitro* drug resistance and response to therapy. Interestingly it causes drug resistance *via* intracellular drug sequestration instead of drug efflux from the malignant cell.

Specific inhibitors of ABC-transporters can sensitize leukemic cells to chemotherapy. For some types of leukemia it would be desirable to develop drugs that inhibit a set of ABC-transporters.

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USE OF MULTIVARIATE MODELS TO IMPROVE PROSTATE CANCER DETECTION

C. Stephan, H. Cammann, M. Lein, M. Schrader, S. Deger, K. Miller and K. Jung

Department of Urology, Charité, Universitätsmedizin Berlin, Germany

Multivariate models including prostate specific antigen (PSA), percent free PSA (%fPSA) and other clinical data such as prostate volume, age, status of digital rectal exam (DRE) have been shown to improve prostate cancer (PCa) detection. This summary gives an overview of PSA and %fPSA-based artificial neural networks (ANNs) and logistic regression (LR) models to reduce unnecessary prostate biopsies. New serum markers like subforms of PSA (*e.g.* proPSA) or other kallikreins show additional value within different models. Recently, the urine marker PCA3, a non-coding RNA, has been shown to detect PCa independently from other markers. Also, the majority of PCa harbour a chromosomal rearrangement that fuses the gene for an androgen-regulated prostate-specific serine protease, TMPRSS2, with a member of the ETS family of transcription factors, most commonly ERG. The addition of PCA3 and gene fusion data within PSA and %fPSA models may further substantially improve PCa detection.

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THE GENE MUTATED IN THE RIDDLE SYNDROME REGULATES A UBIQUITIN-DEPENDENT SIGNALLING CASCADE AT SITES OF DNA DAMAGE

Grant S. Stewart[1], Stephanie Panier[2,3], Kelly Townsend[1], Abdallah K. Al-Hakim[2], Nadine K. Kolas[2], Edward S. Miller[1], Shinichiro Nakada[2], Jarkko Ylanko[2,3], Signe Olivarius[2], Megan Mendez[2], Andrea Tagliaferro[2], Laurence Pelletier[2,3], Nadine Taubenheim[4], Anne Durandy[4], Philip J. Byrd[1], Tatjana Stankovic[1], A. Malcolm R. Taylor[1] and Daniel Durocher[2,3]

[1]Cancer Research UK, Institute for Cancer Studies, University of Birmingham, Vincent Drive, Edgbaston,

Birmingham B15 2TT, UK;
[2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, M5G 1X5, Ontario;
[3]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada;
[4]INSERM U768, Hopital Necker-Enfants Malades, 149, rue de Sevres, 75015-Paris, France

The biological response to DNA double-strand breaks acts to preserve genome integrity. Individuals bearing inactivating mutations in components of this response exhibit a range of clinical phenotypes that include cellular radiosensitivity, immunodeficiency, infertility, progressive neurological dysfunction and cancer predisposition. The archetype for such disorders is ataxia-telangiectasia (A-T) caused by biallelic mutation in *ATM*, a central component of the DNA damage response. Here, we report that the E3 ubiquitin ligase RIDDLIN is mutated in a recently described immunodeficiency and radiosensitivity disorder called RIDDLE syndrome. RIDDLIN acts downstream of RNF8 to orchestrate the accumulation of 53BP1 and BRCA1 at sites of DNA damage. RIDDLIN is itself recruited to the chromatin that surrounds DNA lesions by binding to RNF8-dependent conjugated ubiquitin. Therefore, RIDDLIN and RNF8 define a protein ubiquitination cascade at sites of DNA damage that is important for the overall DNA damage response.

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CELL CYCLE DISTURBANCES AND MITOTIC CATASTROPHES IN CELL LINES FOLLOWING LOW DOSE RATE BETA-IRRADIATION

Torgny Stigbrand[1], David Eriksson[1] ,Theres Lindgren[1], Per-Olov Löfroth[2], Lennart Johansson[2] and Katrine Åhlström Riklund[3]

Departments of [1]Immunology, [2]Radiophysics and [3]Diagnostic Radiology, Umeå University, SE-901 85 Umeå, Sweden

Aims: Antibodies specific for tumour-antigens and labelled with ^{131}I can deliver low dose, low dose irradiation to tumours and cause growth retardation at experimental radioimmunotherapy. The aim was to elucidate the sequential molecular and cellular events occurring in 4 cell lines with different origin that have been exposed to continuous low dose-rate radiation. *Materials and Methods:* Activation of cell cycle checkpoints and mitotic behaviour was investigated in four cell lines: HeLa Hep2, Jurkat, LS174T, and HT29 following low dose irradiation delivered by ^{131}I in cell culture media. Western blots, FACS analysis and immunofluorescence stainings were performed for detection of mitotic aberrations and apoptotic induction. *Results:* A G_2/M arrest was demonstrated by FACS analysis. The G_2/M arrest was transient and the cells reentered the cell cycle still containing unrepaired cellular damage. This premature entry caused an increase of anaphase bridges, lagging chromosomal material and multipolar mitotic spindles as visualised by propidium iodide staining and immunofluorescence staining with a-tubulin antibodies. Furthermore a dose dependent significant increase in centrosome numbers, as well as a dose dependent increase of polyploid cells were detected. These disturbances caused the cells to progress into mitotic catastrophe and a fraction of these dying cells demonstrated apoptotic features as displayed by TUNEL staining 5-7 days following irradiation. *Conclusion:* Low dose rate irradiation was demonstrated to force all four cell lines into mitotic catastrophe and delayed apoptosis. This phenomenon might be important in cell death mechanisms involved in tumour growth retardation following radioiommunotherapy of tumours.

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GENOMIC TOOLS FOR DISSECTING THE LEUKAEMIA GENOME

Jonathan C. Strefford

Cancer Genomics Group, University of Southampton, UK

The detection of chromosomal abnormalities by conventional cytogenetic analysis is an essential component of the multidisciplinary approach to the diagnosis, classification and risk-stratification of patients with leukemia. The clinical utility of cytogenetics has been expanded with the development of fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization (CGH). The latter allows the detection of copy number alterations (CNA) throughout the entire genome in a single reaction. While CGH was capable of highlighting recurrent chromosomal regions and facilitating positional cloning studies, the technique lacked resolution, and it was not until the development of array-based CGH (aCGH) that became possible to determine the gene content of these CNA. Several haematological malignancies have been investigated with this approach and this has significantly advanced our understanding of the molecular mechanisms underlying leukaemogenesis.

We have been using a combination of classical cytogenetics and state-of-the-art genomic profiling technologies to identify novel aberrations in patients with leukaemia. Initially, we performed parallel expression and aCGH profiling to characterized a clinically-relevant chromosome aberration in childhood acute lymphoblastic leukaemia (ALL), termed intrachromosomal amplification of chromosome 21 (iAMP21). With metaphase FISH, including mBAND analysis, we show that iAMP21 is likely the result

of a series of breakage-fusion-bridge cycles. Profiling different patient sub-groups identified evidence of retinoic acid pathway disruption in *ETV6-RUNX1*-positive ALL, and a novel *ETV6*-mediated mechanism of oncogenic activation. With multiplex ligation-dependant probe amplification (MLPA), we have revealed novel methylation target genes in high-hyperdiploidy ALL. By applying FISH, molecular copy number counting (MCC) and breakpoint cloning to patients with unbalanced dicentric chromosomal abnormalities, we reveal *PAX5* disruption as a key molecular event and show how our strategy could be applied to solid tumours, where unbalanced chromosomes are particularly prevalent. By investigating novel *IGH@* partner genes, we show overexpression of members of the *CEBP* family, a finding in contrast to that described in acute myeloid leukaemia (AML).

Here, several examples of novel chromosomal abnormalities in patient with acute leukaemia are described, clearly demonstrating the power of this approach in elucidating molecular events involved in the initiation or maintenance of the leukaemic phenotype. Further studies will surely expand our understanding of cancer pathogenesis and improve the treatment of patients with cancer.

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IMMUNOHISTOCHEMICAL DETECTION AND POSSIBLE PROGNOSTIC VALUE OF CD68 AND KALLIKREIN 6 EXPRESSION IN HUMAN GLIOMA

Tadej Strojnik[1], Rajko Kavalar[2] and Irena Zajc[3]

[1]University Clinical Centre Maribor, Department of Neurosurgery, Ljubljanska 5, 2000 Maribor, Slovenia; [2]University Clinical Centre Maribor, Department of Pathology, Ljubljanska 5, 2000 Maribor, Slovenia; [3]National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Večna pot 111, 1000 Ljubljana, Slovenia

Objectives: CD68 is a marker for microglia. The role of microglia in malignant glioma biology remains unclear. Kallikreins are a subgroup of serine proteases and have recently been strongly associated with tumour progression. Previously, we revealed a strong positive reaction with the CD68 and kallikrein 6 antibodies in most of the cells in a U87 human glioblastoma cell suspension, precultured U87 tumour spheroids and in a rat tumour model. The aim of this study was to evaluate the expression of CD68 and kallikrein 6 in human gliomas, and to investigate the possible prognostic significance. The expression was compared with the expression of some other markers in the same set of glioma patients. *Materials and Methods:* Using immunohistochemical analysis and specific monoclonal antibodies, we evaluated the levels of CD68, cathepsin B, CD31, kallikrein 6 and Ki-67 in histological sections of patients with primary astrocytic tumours. Immunohistochemical scores were determined as the sum of the frequency (0-3) and intensity (0-3) of immunolabeling of the tumour cells. CD68 and kallikrein 6 expression was also analyzed with real-time PCR in 9 brain tumour biopsies. *Results:* Of the 51 patients included, 11 had benign tumours and 40 had malignant ones. A high immunohistochemical score (4-6) for CD68 and cathepsin B was more frequent in malignant tumours than in benign ones, $p=0.036$ and 0.014, respectively. We found higher levels of Ki-67 antigen in the malignant group compared with the benign, $p=0.035$. In contrast, the benign group presented a stronger immune reaction for proteolytic enzyme kallikrein 6 in tumour cells, compared with the malignant group, $p=0.013$. Staining with the CD31 antibody revealed no significant difference in staining of the endothelial cells in malignant *vs*. benign tumours. Univariate survival analysis indicated that imunohistochemical CD68 score above 3 was a significant predictor for shorter overall survival ($p<0.01$). In the malignant group a higher CD68 score (4-6) also indicated worse outcome, although the difference was of boundary significance, $p=0.057$. We confirmed the prognostic significance of cathepsin B in tumour cells. Cathepsin B score above 3 was significant for shorter overall survival ($p=0.04$). Other markers had no prognostic impact. *Conclusion:* Strong immune reaction for CD68 in U87 suspension, spheroids, rat tumour model and also in human malignant glioma samples indicates the important role of CD68 expression in glioma progression. We conclude that specific immunostaining of CD68 and cathepsin B, but not callikrein 6, in tumour cells can be used to predict the risk of death in patients with primary tumours of the central nervous system.

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DRUG RESISTANCE IN CHILDHOOD ACUTE LEUKEMIA AND THE CONCEPT OF LEUKEMIC STEM CELLS

Jan Styczynski

Department of Pediatric Hematology and Oncology, Collegium Medicum, Nicolaus Copernicus University, ul. Curie-Sklodowskiej 9, 85-094 Bydgoszcz, Poland

In spite of the significant progress in pediatric oncology made over last 50 years, still about 20% of children with acute lymphoblastic leukemia (ALL) and 50% with acute myelogenous leukemia (AML) relapse. This may be due to (1) drug resistance, (2) unfavorable cytogenetics, (3) minimal residual disease, (4) presence of leukemic stem cells. Various aspects of drug resistance are different in childhood ALL and AML, as well as in age groups and in comparison to adults

with leukemia. *In vitro* and *in vivo* aspects of the cellular drug resistance profile and mechanisms of resistance to anticancer drugs might have clinical relevance and prognostic value in acute leukemia.

A new concept of cancer stem cells (CSCs) may explain the phenomenon of tumor resistance. CSCs are a population of rare cancer cells exhibiting stem cell properties such as self-renewing, differentiation, tissue reconstitution and multiple drug-resistance. Putative leukemic stem cells (LSCs) have been reported both in ALL and AML.

Several experimental strategies have been utilized to identify and/or isolate putative CSCs: cell surface marker-based analysis, side population (SP) analysis, drug resistance properties, sphere-formation assays, label-retaining properties and microarray technology. Cancer stem cells like normal stem cells share several common features. Side population (SP), as defined by Hoechst exclusion in flow cytometry, represents a small fraction of the whole cell population, expressing high levels of various members of the ABC transporter family (including MDR1 and BCRP), which are responsible for drug resistance. Malignant transformation results from dysregulation of self-renewal pathways. Distinct sets of "self-renewal" genes have been associated with stem cells. The mutations accumulated in normal stem cells could inappropriately activate self-renewal signaling pathways and could potentially lead to cancer.

CSCs are intrinsically drug resistant. In the CSCs model, drug resistance can be mediated by stem cells. Cancer might have a built-in population of drug-resistant pluripotent cells that can survive chemotherapy and repopulate the tumor. Most CSCs have quiescent cell cycle status, reduced sensitivity to cell cycle-dependent agents, express ABC transporters, cause drug efflux, have an active DNA-repair capacity and a resistance to apoptosis. Identification of phenotype and molecular properties of CSCs enable development of the best strategies to target CSCs (supported by grant MNiSW N407 078 32/2964).

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ANTITUMOR ACTIVITY OF CURCUMIN IN A HUMAN NON-SMALL CELL LUNG CANCER XENOGRAFT MODEL

Chin-Cheng Su, Guang-Wei Chen, Jai-Sing Yang, Chang-Lin Wu, Chi-Cheng Lu and Jing-Gung Chung

Division of General Surgery, Department of Surgery, Buddhist Tzu Chi al Hospital, General Hospital, Hualien; Traditional Chinese Medical Department, Chung-Ho Memorial Hospital, Kaohsiung Medical University, Kaohsiung; Departments of Chinese Medicine Resources, Pharmacology, Biological Science and Technology and Microbiology, China Medical University, Taichung 404, Taiwan, ROC

Curcumin (diferuloylmethane), a phenolic compound from the plant *Curcuma longa* (Linn.) has been shown to exhibit antitumor activity, such as induced cell cycle arrest and apoptosis, in many human cancer cell lines, including lung and liver cancer cells *in vitro*. However, the efficacy and *in vivo* mechanism of action of curcumin in human lung cancer cells have not been well investigated. In this study, we assessed the *in vivo* therapeutic effects of curcumin on lung cancer cells. In a primary study, we used MTT assay and determined that curcumin inhibits proliferation of H460 lung adenocarcinoma cells. Furthermore, the cancer cells showed increased levels of activated caspase-3 and an increased ratio of Bax/Bcl-2, suggesting that the cells were undergoing apoptosis. At the same time, cell cycle analysis showed that there was an increased number of cells in the G_2/M phase. For *in vivo* studies, female athymic Nu/nu mice were xenografted with H460 tumors and on day 4 onwards curcumin was administered orally at dose of 300, 450 and 550 mg/kg/day for 24 days. As a control, xenografted tumors were separately treated with docetaxel (10 mg/kg *i.v.* bolus on day 5, 11, 17, 23). The tumor volumes and animal body weight were measured every three days. Expression of PARP, Bcl(2), Bax, and caspase-3 families of proteins was measured by Western Blotting (WB), while TUNEL and immunohistochemical methods were utilized to determine DNA fragmentation and cleaved caspase-3 levels respectively. Curcumin inhibited growth of H460 cells and caused apoptosis as evidenced by nuclear condensation in treated H460 cells.

Curcumin caused 38 and 63% reduction in the xenografted tumor volumes at a dose of 100 ($p<0.05$) and 300 ($p<0.01$) mg/kg/day, respectively, when compared to controls. Curcumin-dependent suppression of xenografted tumor growth involved up-regulation of PARP, Bax, caspase-3 and repression of Bcl(2) expression thus suggesting induction of apoptosis by a mitochondrial pathway. In conclusion, our studies suggest the potent antitumor activity of curcumin against NSCLC cells. Thus, curcumin may be a promising novel chemotherapeutic agent for the treatment of human lung cancer.

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MOLECULAR MECHANISM OF THE PROGRESSION FROM PRE-NEOPLASTIC GROUND GLASS HEPATOCYTES TO HEPATOCELLULAR CARCINOMA: IMPLICATION FOR CHEMOPREVENTION AND TARGETED THERAPY OF HCC

Ih-Jen Su, Juei-Chu Yang, Hang-Chieh Wu and Wen-Ya Huang

Division of Clinical Research, National Health Research Institutes, and Graduates Institutes of Basic Medicine and Biotechnology, Tainan, Taiwan, ROC

The discovery of ground glass hepatocytes (GGH) that contain hepatitis B virus (HBV) surface antigens by Hadziyannis and Popper in 1972 represents a historic landmark in the pathology of chronic HBV infection. Different types of GGHs have been correlated to the expression patterns of core/surface antigens and stages of virus replication. The original two types (designated Type I and II) of GGHs were found by us to contain specific pre-S mutants, with deletions over the pre-S1 or pre-S2 regions, respectively. Both types of pre-S mutants are accumulated in endoplasmic reticulum (ER) and induce ER stress response, oxidative intermediates and DNA damage. Type II GGHs consistently harbor pre-S2 mutants, which may escape from immune attack and grows preferentially into clusters in the liver, usually of advanced-stage disease. The pre-S2 mutants, albeit inducing a weaker level of ER stress response than pre-S1 mutants, could additionally initiate ER stress-independent JAB1/p27/RB/cyclin A, D signals to initiate proliferation of hepatocytes, explaining the clustering of Type II GGHs. Recently, we further demonstrated that there is activation of the VEGF/Akt/mTOR signal pathway in GGH, the precursor neoplastic lesion, and in the early-stage livers of 3 to 6-month-old transgenic mice harboring pre-S2 mutant which develops into HCC at 2 years of age. The prevalence of pre-S mutants in chronic HBV carriers also carries a higher risk of developing HCC or recurrence after HCC resection. Combining these data, we conclude that GGHs, particularly Type II GGH, represent pre-neoplastic lesions in chronic HBV infection. Targeting the Akt/mTOR pathway or JAB1/RB pathway by natural products or drugs may provide prevention for the development or therapy of HCC in chronic HBV carriers.

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THE BCL2 MAJOR BREAKPOINT REGION (MBR) WITHIN THE 3'-UNTRANSLATED REGION REGULATES GENE EXPRESSION

C. Ma[1,2], J. Zhang[1], L.K. Durrin[4], J. Lv[1], F Gong[1], L. Sun[1], N. Yang[1,2], D. Zhu[3], X. Han[1] and Y. Sun[1,2]

[1]Key Laboratory of Human Functional Genomics of Jiangsu Province, [2]Department of Cell Biology and Medical Genetics, [3]School of Pharmacy, Nanjing Medical University, Nanjing, PR China and [4]Department of Molecular Medicine, City of Hope National Medical Center, Duarte, CA, USA

BCL2 is a key regulator of apoptosis and has additional roles in cell cycle control, differentiation and DNA damage repair. Disrupted expression of BCL2 has been implicated in a wide variety of human cancers, including breast, pancreas, colorectal, lung, and both acute and chronic leukemias. Diverse mechanisms are likely to account for the dysregulation of BCL2 gene expression in these different neoplasms. The 279-bp major breakpoint region (mbr) within the 3'-untranslated region (3'-UTR) of BCL2 gene is a hot breakage spot in t(14;18) (q32;q21) chromosome translocation occurring in follicular lymphoma. The mbr is also a matrix attachment region (MAR). A special AT-rich sequence binding protein 1 (SATB1), a MAR binding protein, binds to the mbr. SATB1 regulates multiple genes during development and also sequesters chromatin remodeling proteins and transcription factors to MAR elements. In vivo mbr binding by SATB1 strongly implicates that this BCL2 region could be involved in BCL2 regulation. In this study we first explored the regulatory function of the mbr in BCL2 transcription and its correlation to SATB1. We demonstrated that the mbr within the 3'-UTR of BCL2 gene upregulated the reporter gene expression. Deletion of the mbr by homologous recombination significantly decreased the transcriptional activity of the corresponding allele in the mbr-/mbr+ heterozygous cells. Furthermore, the BCL2 allele deleted of the mbr had a slower response to apoptotic stimuli than did the wild-type allele, implying the mbr is required for regulation of the BCL2 gene in response to apoptosis stimulation. The regulatory function of the mbr was mediated through SATB1. Overexpression of SATB1 increased BCL2 expression, while knockdown of SATB1 with RNAi decreased BCL2 expression. These observations clearly indicated that the mbr could positively regulate BCL2 gene expression and this regulatory function was dependent on SATB1.

To explore the mechanism underlying the mbr regulatory function, we began to test our hypothesis that the mbr regulates the BCL2 gene by forming a special chromatin loop structure through SATB1. Based on bioinformatics analysis five SATB1 binding sequences have been identified upstream of the BCL2 promoter using EMSA and ChIP assays. 3C analysis revealed that two of them had specific interaction with the mbr, indicating that BCL2 gene could form chromatin loop between mbr and promoter region by anchoring its base to SATB1. Additional experiments are undergoing to correlate the chromatin loop structure to the regulation of BCL2 transcription. Our present work provides an excellent opportunity to study the interplay of protein interactions and DNA sequence determinants in the function of a MAR associated with a locus critical for programmed cell death and carcinogenesis.

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ABERRANT NOTCH SIGNALING IN ACUTE LEUKEMIA

Kazumi Suzukawa, Noriko Nemoto and Shigeru Chiba

Department of Clinical and Experimental Hematology, Major of Clinical Medicine, Graduate School of

Comprehensive Human Sciences, University of Tsukuba, Tennoudai, 1-1-1, Tsukuba, Ibaraki 305-8575, Japan

Notch signaling influences cellular proliferation, differentiation and apoptosis in diverse biological processes. Consistent with diverse effects of Notch signaling in multiple tissues, abnormalities of Notch pathway molecules are associated with malignant neoplasms. In humans, these include gain-of-function mutations of *NOTCH1*, the gene for a receptor, and loss-of-function mutations of *FBW7*, the gene for an E3 ubiquitin ligase, in T-cell acute lymphoblastic leukemias (T-ALL), and generation of a fusion gene involving a chromatin regulator gene *MLL* and a Notch-specific coativator gene, *mastermind like-2* (*MAML2; MLL-MAML2*), in T-ALL as well as acute myeloid leukemias. Results of luciferase assay revealed that the *MLL-MAML2* fusion gene caused aberrant Notch signaling. Interestingly, *MLL-MAML2* conferred interleukin-3 (IL-3)-independent growth on cells that were originally dependent on IL-3 through the activation of *IL-3* gene transcription. Activation of an autocrine cytokine circuit might be one of the mechanisms of leukemogenesis by *MLL-MAML2*.

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NEW CONCEPTS ON RISK FACTORS OF HPV AND NOVEL SCREENING STRATEGIES FOR CERVICAL CANCER PRECURSORS

Kari Syrjänen

Department of Oncology and Radiotherapy, Turku University Hospital, Turku, Finland

During the past several years, the author has been engaged in co-ordinating two major multi-centre trials testing optional screening tools for cervical cancer (CC) in low-resource settings both in East Europe and in Latin America. These international trials include the NIS (New Independent States of the former Soviet Union) cohort (n=3,187 women) and the LAMS (Latin American Screening) study (n=12,114 women). In both studies, a sizeable cohort of women (887 and 1,011, respectively) have been prospectively followed-up to assess the natural history of high-risk human papillomavirus (HR-HPV) infections and the role of implicated risk factors as potential predictors of disease outcome (acquisition, persistence and clearance).

We will discuss some of the key observations recently reported from the NIS and LAMS studies, with special emphasis on i) risk factors that are still controversial (*i.e.*, oral contraception, OC; and smoking) or not previously studied (drug addiction); ii) reproductive factors as potential co-factors of HPV infections in cervical carcinogenesis (*i.e.*, age at menarche, menopause); and finally on iii) the performance of different screening strategies among young and older women.

The NIS Cohort failed to establish OC as a risk factor of CC. In all future studies, the strong confounding from the life-style behavioural factors must be taken into account while interpreting the data on OCs as potential risk factors of CC. Similarly, it now seems that the increased risk (if any) of CC among smokers seems to be attributed to the increased acquisition of HR-HPV infections, of which the smoking status is an independent predictor in a multivariate model. The same seems to apply to drug addiction as a risk factor of CC. The recent LAMS data show that drug abuse itself is not a risk factor of i) contracting HR-HPV infection, or ii) developing high-grade CIN. Instead, drug abuse seems to be closely associated with several of the indicators of risky sexual behavior, which predisposes women to oncogenic HPV infections and thus indirectly contributes to the development of CIN2+ lesions.

Data from the NIS Cohort clearly implicate that menarche age is not associated with increased risk of HR-HPV infection, or development of high-grade CIN, feasibly explained by the fact that menarche age does not have any effect on the outcome of CIN lesions or HR-HPV infections in a longitudinal setting. Another special group are post-menopausal women, recently shown to have a second peak of HR-HPV prevalence in many populations. The NIS Cohort data suggest that among women who fail to eradicate their HR-HPV infection by the menopause, there is i) a transition from multiple infections to single-type infections, and ii) selection of an integrated viral clone already taken place, driving the process towards an aggressively progressing cervical disease.

Finally, these special features of HR-HPV infections among young and elderly women lead us to consider whether different screening strategies should be needed for younger and older women. Concordantly with other recent reports, data from the LAMS study show that conventional Pap and HC2, but not LBC and VIA, perform significantly differently among younger and older women. However, the choice of optimal screening test for young and older women depends on whether the highest positive predictive value (PPV) (Pap test) or the best balance between sensitivity and specificity (SE/SP) (HC2) is used as the selection criteria.

Both the NIS Cohort and LAMS Study have significantly contributed to solving several of the open issues in the natural history of HR-HPV infections as well as in sorting out the optional screening strategies in low-resource settings and for women of different age groups. In the long term, it is most likely the cost-effectiveness that is the decisive factor of which screening test will be selected. Needless to say, screening for cervical cancer precursors will be mandatory in the foreseeable future, even in this emerging era of prophylactic HPV vaccination.

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CHLOROPHYLL *b* REVERSES MULTIDRUG RESISTANCE OF CANCER CELLS

M. Szabo[1], L. Tanacs[2], I. Ocsovszki[3], P. Molnar[4] and J. Molnár[5]

[1]Department of Plant Biology, [2]Department of Chemistry, Faculty of Engineering, [3]Institute of Biochemistry, University of Szeged, Szeged, [4]Institute of Pharmacognosy, University of Pécs, Pécs, [5]Institute of Medical Microbiology and Immunobiology, University of Szeged, Hungary

Various plant compounds and metabolites have been identified and described as potential anticancer agents or modifiers of multidrug resistance. Among these active compounds there are chemicals, like carotenoids and terpenoids. The aim of this study was to evaluate the effect of chlorophylls freshly isolated from the leaves of bean plants. The effects of chlorophyll a and b were studied on the drug accumulation in human MDR1 gene transfected mouse lymphoma cells. Chlorophyll *a* and *b* had a similar dose dependent effect on cell membrane structure without altering the cell size measured by flow cytometry.

Chlorophyll *b* was able to elevate moderately Rhodamine 123 accumulation of MDR tumor cells. In addition the combination of chlorophyl *b* and capsorubin showed a remarkable increase in the inhibition of Pgp 170 while the chlorophyll a reduced the effect of capsorubin.

MDR reversal effect of chlorophyll *b* can be explained by energetically favorable electron charge transfer complex formation between chlorophyll *b* and the carotenoid pigments on the Pgp170. The energy gradient is in the optimum range from carotenoid to chlorophyll *b*, but low binding energy of chlorophyll *a* does not modify the functionally active conformation of the Pgp 170 membrane protein. In a checkerboard experiment the combination of doxorubicine and chlorophyll *b* resulted in a synergistic interaction on inhibition of proliferation of MDR tumor cells *in vitro*. The MDR cells were re-sensitized to the antiproliferative effect of anticancer drug, namely to doxorubicine.

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QUESTIONS OVER HEAD AND NECK MALIGNANCIES AND ADVANCES ON BIOLOGICAL TUMOROLOGY (UNDERSTANDING, TREATMENT AND FOLLOW UP)

György Szalai[1], Joseph Molnar[2] and Peter Grandics[3]

[1]MONMED, Budapest, 1056-, Hungary;
[2]Institute of Medical Microbiology and Immunobiology, University of Szeged, Hungary;
[3]A-D Research Foundation 5922 Farnsworth Ct, Carlsbad, CA 92008, USA

Over half a million malignant tumors of the head and neck are diagnosed each year worldwide, about 4,000 of them in Hungary. Nine tenth of them approximately are cancers of squamous cell origin and first diagnosed at stage III or IV. An overall 5-year survival rate is below 50% for nearly three decades. Current therapies on selected patients permit organ preservation. Despite aggressive combination of chemotherapy with CDDP and 5-FU and external-beam radiation therapy, local and regional recurrence is 30% and distant recurrence occurs in 25% of the patients. The high mortality and morbidity encourage the pursuit alternative therapeutic strategies. The immune system of advanced stage head and neck cancer (HNC) patients is frequently suppressed. Poor immune function has been correlated with poor clinical outcome. We have linked the development of cancer to infection(s) during which antigenic determinants from pathogens mimicking self-antigens are co-presented to the immune system, leading to breaking T-cell tolerance. Some level of autoimmunity is normal and necessary for effective pathogen eradication. However, autoreactive T-cells must be eliminated by apoptosis when the immune response is terminated. Apoptosis can be deficient in the event of a weakened immune system, the causes of which are multifactorial. Some autoreactive T-cells suffer genomic damage in this process. The resulting cancer stem cell still retains some functions of an inflammatory T-cell, so it seeks out sites of inflammation inside the body. Due to its defective constitutive production of inflammatory cytokines and other growth factors, a stroma is built at the site of inflammation similar to the temporary stroma built during wound healing. The cancer cells grow inside this stroma, forming a tumor that provides their vascular supply and protects them from cellular immune response. Immunotherapeutic strategies have been previously attempted in an effort to enhance immune function and improve survival. Covalently linking proteins and cytokines could have enormous potential for the *in vivo* manipulation of the immune system. Human carcinoembryonic antigen (CEA) is an oncofetal glycoprotein over-expression of which by gastrointestinal carcinomas is well known. Expression of CEA in HNC is not widely recognized. It is important to note that most of these studies used polyclonal antibodies that may have cross-reactivity with CEA-related antigens. Recent studies evaluated CEA in preclinical and clinical levels as a target for specific immunotherapy against HNC. Follow up CEA and HNC correlations by monoclonal antibody presented positive at mRNA, CEA-protein and in tumor lysates, too. Because cell-surface expression of CEA is low in the SCC cells and strong cytoplasmic staining that direct research show a possible

way to a vaccine mediated immunotherapy against HNCs. Over the past 10 years great amount of research and clinical data have appeared from many organizations but many challenges remain. Evidence based medicine, research and integration of data directly into approaches based on mathematical modeling. Case studies will be presented.

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KRN951, A HIGHLY POTENT AND SELECTIVE PAN-VEGFR TYROSINE KINASES INHIBITOR

E. Taguchi, K. Takahashi, Y. Tazunoki and T. Miura

Development Research Laboratories, Kirin Pharma Co., Ltd., Takasaki, Gunma 370-1295, Japan

Sustained growth of solid tumor is dependent on angiogenesis. Vascular endothelial growth factor (VEGF) and its receptors are promising targets for antitumor therapy because of its major role in the tumor angiogenesis. We discovered KRN951, a novel orally active inhibitor of VEGF receptor tyrosine kinases, and elucidated its *in vitro* and *in vivo* characteristics. KRN951 potently and specifically inhibited *in vitro* VEGF-stimulated phosphorylation of all three critical VEGF receptors VEGFR-1, -2 and -3, at picomolar concentrations, while it inhibited c-Kit and PDGFR at 10-times higher concentrations. This inhibition profile of KRN951 contrasts with other approved oral antiangiogenic drugs; they inhibit not only VEGFRs at naomolar level but also other kinases, such as c-Kit, PDGF, Raf and so on. Because of multi-target inhibition activity in these drugs, various toxicities are seen in humans. In contrast, due to the high specificity of KRN951, it is assumed that KRN951 may have a mild toxicity profile in humans. KRN951 also showed inhibitory activity against VEGF-driven, mitogen-activated protein kinases and the proliferation of endothelial cells at picomolar concentrations, but affected neither FGF-driven nor EGF-driven cellular responses even at 100 nM. KRN951 did not show antiproliferative effects *in vitro* on various tumor cells.

Daily oral administration of KRN951 in athymic mice and rats resulted in significant growth inhibition of various types of human tumor xenografts at extraordinarily low doses without obvious toxicity. Effective doses of KRN951 that exerted significant inhibitory activity on tumors were 5 mg/kg/day in nude mice and 0.2 mg/kg/day in nude rats. Significant inhibitory activity on tumors were also exerted in combination with cytotoxic drugs, without additional toxicity. In a syngeneic peritoneal disseminated tumor model, KRN951 suppressed intraperitoneal tumor growth and accumulation of ascites, and consequently prolonged survival. KRN951 also normalized the architecture of tumor-induced neovasculature with aberrant structure. In newly established multiple myeloma xenograft model, KRN951 also efficiently inhibited tumor-dependent osteolysis of bone marrow. Noteworthy was the superior efficacy of KRN951 over bevacizumab against tumor-associated onset of hind leg paralysis at equivalent survival benefit doses.

In conclusion, it was demonstrated that KRN951 is a highly potent and selective triple-VEGFR inhibitor associated with *in vivo* antitumor activity in wide variety of tumor models. The highly potent, specific unique profile of KRN951 will allow it to be easily combined with other agents at low doses and suggests the potential for a superior therapeutic index beyond the current multi-targeting tyrosine kinase inhibitors. Encouraging results of a Phase I clinical trial will also be presented.

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A NOVEL MULTIMODALITY TREATEMENT FOR PANCREATIC CANCER

Hiroshi Takamori, Hiroshi Tanaka, Yasuo Sakamoto, Yoshiaki Ikuta, Osamu Nakahara, Satoshi Furuhashi, Toru Beppu, Masahiko Hirota, Keiichiro Kanemitsu and Hideo Baba

Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan

Background: Patients with pancreatic cancer often suffer from tumor recurrence despite curative resection, which indicates that systemic therapy added to local control might be required to cure pancreatic cancer. The aim of this study was to assess the feasibility and response to a novel multimodality therapy composed of pancreatic resection and intraoperative radiation therapy (IORT) combined with pre- and post-operative chemotherapy of 5-Fluorouracil (5-FU) intra-arterial continuous infusion and systemic gemcitabine administration for pancreatic cancer. *Patients and Methods:* Forty-two patients with potentially resectable pancreatic cancer underwent this multimodality therapy. Enroll criteria were: i) age<80, ii) PS<1, iii) no evidence of distant metastases, iv) no evidence of tumor extension to the celiac axis or the superior mesenteric artery, v) major organ function preserved. For preoperative chemotherapy, 5-FU was administered at a dose of 125 mg/m^2/day on days 1-5 every week as a continuous pancreatic and hepatic arterial infusion, and gemcitabine was infused intravenously at a dose of 1000 mg/m^2 for 30 min once weekly. Pancreatic resection combined with IORT (30 Gy, 12 Mev of electron beam) was performed after a one-week rest following the completion of preoperative chemotherapy. Postoperative chemotherapy was performed in the same way as

preoperative chemotherapy after the recovery from surgery. *Results:* In preoperative chemotherapy, the most common toxicities of grade 3/4 were hematological events including neutropenia (15 pts.), leukocytopenia (7 pts), and thrombocytopenia (2 pts). Only one patient experienced a delay in surgery because of preoperative chemotherapy-related neutropenia. All 42 patients underwent surgery. In postoperative chemotherapy, grade 3/4 toxicities included neutropenia (22 pts.), leukocytopenia (13 pts), anemia (4 pts.), thrombocytopenia (3 pts), liver abscess (3 pts.), cardiac ischemia (2 Pts.), perforation of small intestine (1 pt), and renal failure (1pt), although no chemotherapy-related death was observed. The median follow-up period was 26.2 months. The 1-year, 3-year and 5-year overall survival rates were 79.9 %, 54.8% and 30.4%, respectively, with MST of 36.5 months. *Conclusion:* This new multimodality treatment is feasible and tolerable, and may contribute to further improve the survival of patients with pancreatic cancer.

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EXPRESSION ANALYSIS TO IDENTIFY GENES INVOLVED IN ACQUIRED RESISTANCE TO CISPLATIN IN OSTEOSARCOMA CELLS

Koutarou Takazawa[1], Hiroyuki Tsuchiya[1], Ueda Yoshimichi[2], Yoshimitsu Kanazawa[1], Sadao Ii[1] and Katsuro Tomita[1]

[1]Department of Orthopaedic Surgery, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa;
[2]Department of Pathology, Kanazawa Medical University, 1-1 Uchidana-machi, Kahoku-gun, Ishikawa, Japan

Background: Clinical observations indicate that tumour cells can acquire tolerance when an anticancer drug is administered repeatedly. Gene expression in cisplatin-resistant cells was analysed to identify changes in gene expression particularly early in the course of cisplatin exposure. *Materials and Methods:* After establishing a cisplatin-resistant human osteosarcoma subline (OST/R) and establishing two additional sublines by more brief repeated exposure, cDNA expression microarrays were used to study genes linked with prolonged exposure to cisplatin of human cancer cells. *Results:* OST/R cells showed increased expression of 17 genes and reduced expression of 14. Genes associated with DNA repair, apoptosis, cell cycle progression, and proliferation were associated with the acquired resistance. Genes showing early changes were also identified. *Conclusion:* Identification of genes showing altered expression in the early stages of development of resistance to cisplatin may help to improve the therapeutic effectiveness of this drug.

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SURVIVIN EXPRESSION IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA; ITS PROGNOSTIC IMPACT AND SPLICE VARIANT EXPRESSION

Shinsuke Takeno, Shin-ichi Yamashita, Kiyoshi Ono, Kentaro Anami, Keita Tokuishi, Mirei Kamei, Shuji Suehiro, Michiyo Miyawaki, Ei-ichi Tanaka, Masao Chujo, Satoshi Yamamoto and Katsunobu Kawahara

Department of Oncological Science (Surgery II), Oita University Faculty of Medicine, Oita, Japan

Purpose: The present study examined the clinicopathological impact of survivin expression in esophageal squamous cell carcinoma (ESCC). In addition, the biological role of anti-apoptosis parameters in ESCC was examined immunohistochemically. *Patients and Methods:* Subjects comprised 71 patients followed for 5 years after surgery for ESCC and analyzed immunohistochemically to examine the clinicopathological impact of survivin expression. Separately, 37 fresh frozen samples of ESCC obtained recently were examined concerning splicing variant expression of survivin using reverse-transcription polymerase chain reaction (RT-PCR). *Results:* Immunohistochemical survivin expression was detected in nuclei of 10 ESCC specimens (14.1%) and cytoplasm of 22 specimens (31.0%). Nuclear expression displayed no clinicopathological implications, but cytoplasmic expression correlated with histological differentiation (p=0.002) and tumor invasion (p=0.073), and showed prognostic impacts in uni- (p=0.0184) and multivariate (p=0.0299) analyses. Survivin, survivin-2B and survivin-deltaEx3 mRNA were amplified in 31 (83.8%), 23 (62.2%) and 26 (70.3%), respectively, by RT-PCR. Survivin-2B level correlated significantly with histological differentiation (p=0.038), but no other significant correlations were identified between any mRNA and clinicopathological factors. *Conclusion:* As a molecular biological anti-apoptotic factor, survivin expression was of use in assessing clinical outcomes in ESCC. Inhibition of survivin may be useful as a molecular biological therapy in ESCC.

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BIO-IMMUNOTHERAPY, A COMBINED BIOLOGICAL AND IMMUNOLOGICAL CANCER THERAPY MODALITY

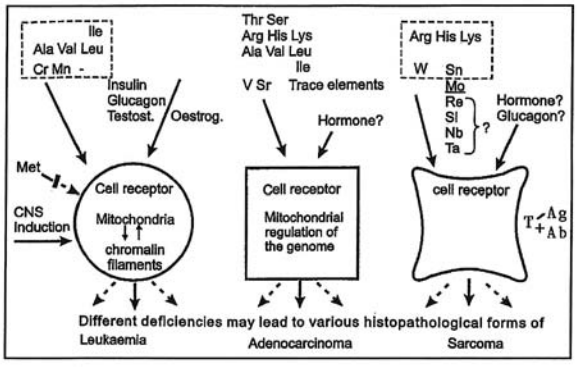
Thomas Tallberg and Jan Dabek

The Institute for Bio-Immunotherapy, Helsinki 00200 Finland

Tumours may spontaneously regress which implies that mammals possess a natural intrinsic regulatory capability to

control the de-differentiation of specialized organ cells, "The metabolic triumph of the host" (Dr. W.M. Cole 1974). Thus a novel paradigm is that cancer represents a complex chronic metabolic deficiency disease which can be compensated by biological dietary means. The aim was consequently to analyze if the disturbed interior milieu can be corrected/compensated for leading to a biological cure without side-effects. In randomized clinical studies with hundreds of cancer patients, over 30 years, highly significant (p=0.001) improvement in the survival rate has been obtained by feeding them a specific combination of nutritional amino acids, trace-element ions, inductional central nervous system lipids (CNS), thus supporting hormonal balance and specifically activating the patients autologous immune-defence. The role of these natural aetiological biomodulating factors regulating three major forms of cancer: leukaemia, adenocarcinoma, and sarcoma, have primarily been outlined (Figure).

Hypothetical cell-control mechanism for steady-state function exerted by amino-acid / trace-element pentamer complexes mediated by hormones



Selective deficiencies in these pentamer codes may be expressed as various histopathological forms of cancer. Dietary restriction of methionine is prescribed, since it seems to be a growth factor for leukaemic stem cells.

Significantly better disease-free intervals were achieved with long-term use of powders containing these vital dietary supplements to correct the complex aetiological metabolic deficiency causing cancer. In a randomized study with 127 patients suffering from metastasized renal cancer, dietary supplements were also able to arrest recurrent disease. Pertinent bio-modulating dietary components were: L-amino acids: Ala, Arg, Asp, Lys; the trace-elements: Cr, Mo, Se, Sn, V; central nervous system (CNS) lipids, and physiological doses of vitamins. Significantly improved clinical results were also obtained with cutaneous (102 cases) and uveal melanoma (54 patients) ingesting Gly, Glu, Ala, Asp, Ile, Lys, Cr, Se, Sn, V, W, and CNS-lipids.

Ready-made powders made to treat prostate cancer (decreasing Gleason scores) are available from our Institute, at a cost of only 2-4 €/ day (Int J Biotechnology, *9*: 3/4; 391-410, 2007). In bio-immunotherapy, significant clinical improvement has been achieved from dietary correction alone in renal cancer (p=0.04), and high risk (T3) uveal melanoma (p=0.001), but clinical results are usually further improved if the therapy is combined with active specific immunotherapy utilizing polymerized autologous tumour tissue (except for prostate cancer). Tumour tissue should therefore always be saved at surgery to facilitate the preparation of individual vaccines since a patient's malignant cells contain a fingerprint of antigenic tumour markers (J Biol Chem, *242*: 1651-1659, 1967). Similar good therapeutic results have also been obtained with other forms of cancer (J Austr Coll Nutr & Env Med, *22(1)*: 1-20, 2003). This healing reaction does not involve apoptosis or lysis of tumour cells as they regain normal healthy function, with complete regression (CR) even of large tumours, without a scar. Regular immune reactions do not have such a capacity. Actually activated regulatory organ-specific mitochondria have in fact been found to be involved in the healing process. Metabolic bio-modulation can also prevent recurrent cancer because it actively strives to correct the aetiological deficiency. Our standard therapy primarily related only to symptoms of this metabolic deficiency disease. It is as if only the loose teeth of a scurvy patient were removed instead of giving him vitamin-C. Malignant transformation caused by genetic weaknesses (*e.g.* HNPCC) is out of reach for gene therapy, since it involves aberrations in more than three genes, but malignant transformation can be prevented by dietary supplementation administering small amounts of pertinent essential metabolic components aimed at regaining the physiological internal milieu in the patient's body. Healthy persons attain this balance from their normal diet. All bio-modulating components involved are natural substances, and thus ethical, inexpensive, easy to administer, with a long shelf life. Bio-immunotherapy entails no side-effects, but does have also prophylactic potential.

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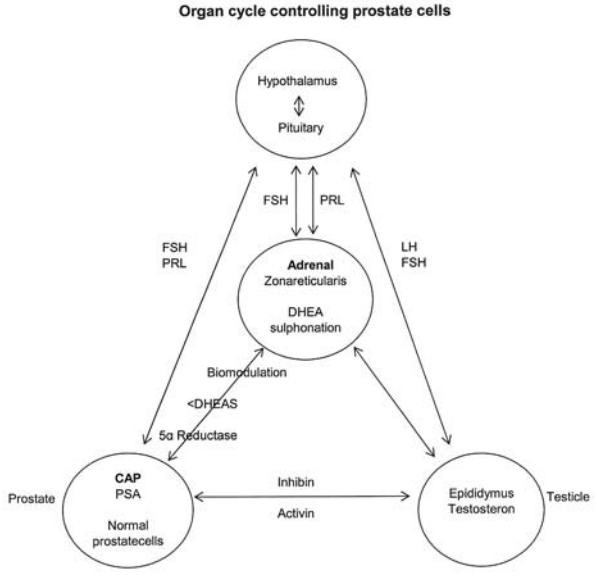
PROSTATE CANCER, AETIOLOGICAL, THERAPEUTIC, PROGNOSTIC AND PROPHYLACTIC FACTORS

Thomas Tallberg and Mervi Dabek

The Institute for Bio-Immunotherapy, Helsinki 00200 Finland

In the eighties, prostate cancer (PCa) was justly considered a hormone-dependent disease (Huggins & Hobbs 1941), but was lacking curative treatments and a comprehensible aetiology. Several signs for adrenal involvement coupled with metabolic factors impelled this analysis on its aetiology. PCa seems to stem from a deficient production of two neuroendocrine components produced by adrenal zona-reticularis cells (ZR). One increases FSH, the other prolactin

(PRL) levels. A curative ZR feed-back reaction can be activated in PCa patients by dietary supplementation utilizing ready-made powders (for 2€/day) compensating the aetiological metabolic deficiency. Natural components prescribed are: amino-acids serine (Ser), arginine (Arg); trace-element ions, strontium (Sr) and vanadium (V), in addition to ingestion of vital central nervous system, CNS-lipids, as canned "Neurofood" Ltd. Helsinki (cans of 220g) mixed with fruits for the sake of taste. The full recipe for these powders is published in Int J Biotechnology, *9*: 3/4, 401, 2007. This non-invasive biological treatment schedule specifically activates an adrenal feed-back cycle controlling prostate cells (Figure).



Disease progression is arrested, ultimately curatively, evidenced as complete regression of multiple bone metastases (CR >17 years). Levels of PSA - a serine protease –may become stable or regress since the ingested substrate (Ser) causes enzyme inhibition. Gleason scores may decline from 8 to 4, paired with reduced urinary distress. PCa found incidentally, or by screening should primarily be treated utilizing dietary supplementation since PSA may decrease in a dose-response manner, whereby serious side-effects caused by invasive treatments could be avoided. A good prognosis is usually registered as increased FSH, PRL and SHBG levels, declining DHEAS and PSA. In patients with metastases, or initially a PSA exceeding 15 ng/ml, intermittent total androgen ablation therapy (for only 10 days) is prescribed in synergy with continuous dietary supplementation using PCa powders to sustain the adrenal ZR feed-back reaction. We prescribe only Zoladex (3.6 mg) implants shielded by Androcur 50 mg x 2/day for ten days around the LHRH injection. Androgen ablation intervals vary from 2-24 months, based on patient response permitting time for the adrenal feed-back reaction to function. This bio-modulating schedule has been sustained already for decades without emergence of a hormone refractory state (HRPC). An increase in FSH is strived for preferably reaching in excess of normal levels (>10 IU), while PSA may remain normal or also increase before the next hormone treatment. Androgen ablation should not attain a PSA nadir since excessive androgen suppression also decreases FSH to dangerously low levels (<1 IU) when the adrenal feed-back cycle has been exhausted, instigating HRPC. This fatal exhaustion is not due to pituitary dysfunction since PRL is then markedly increased in patients. Watchful waiting, even performed as active surveillance omitting any effort to prevent the disease progression is equivalent to Russian roulette. A rare form of PCa is diagnosed from soft tissue metastases and their activin levels are excessively increased while inhibin stays low, but patients respond positively to our bio-modulating treatment. Orchiectomized patients have immeasurable inhibin, although normal activin levels as in pregnant females or ladies on oestrogen substitution therapy. There is a new incentive for screening since PCa in the early phase can be arrested by non-invasive dietary means alone. Improved diagnostic serum markers such as EPCA-2, kinases, PSA velocity, coupled with MRI and constructive dietary trials inciting ZR feed-back reactions, should diminish the need for 12 biopsy cores, as the latter may actually spread malignant cells effecting even a higher incidence of recurrent PCa, than the already disturbing incident of >35%.

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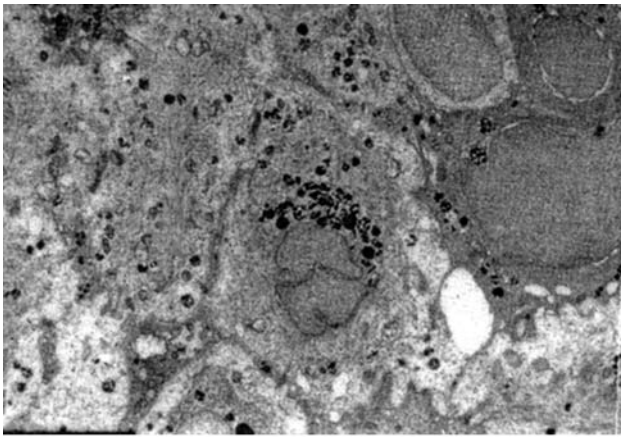
CANCER REGULATED BY ORGAN-SPECIFIC MITOCHONDRIA *VIA* LIPIDOMICS, GENOMICS AND PROTEOMICS

Thomas Tallberg and Raija Hallamaa

The Institute for Bio-Immunotherapy, Helsinki 00200 Finland

The observation was made in 1975 that in the serum of cancer patients there could transiently appear some of the billions of vital lipids, the "lipidome", in the central nervous system and spinal cord (CNS). Lipidomics depicts studies on neurobiology describing the clinical and psychodynamic function of these billions of vital lipids contained in our brain, articulated as harmonious synaptogenesis. The aim of our study was to relate certain clinical features of this immensely complex panel of neurological functions synchronized by this multifaceted brain-lipid network. In embryogenesis construction of a functional CNS is linked to organ-specific mitochondria – present especially in brain capillaries. Our daily mental function and stress consumes certain of these vital CNS-lipid molecules resulting in

various clinical psychological and stress conditions. Healthy reconstruction of this physiologic consumption of CNS-lipids proceeds during our sleep providing that our serum contains certain vital CNS-lipid components supplied *via* our diet. Blood-brain barrier lesions causing a leaking of essential vital lipids into blood could tilt the inductional control exercised by the injured axon, and new malignant cells could appear in the affected enervated area. Dietary supplementation utilizing vital, prion-free CNS-lipids can restore normal cell induction, improve the motor and mental balance of patients, and led also to physiological therapeutic effects in cancer patients. A short survey of these CNS entities will be presented. The human genome project revealed the surprising nucleotide sequence analogy in chromosomes of mammalian species. Consequently mitochondrial mtDNA must have been involved in constructing the chromosomes as a "memory of evolution". Random mutations would exclude such interspecies nucleotide analogies. Mitochondria produce energy for the nucleus but also regulate the genomes they have created over eons of their phylogenetic toil (Trends Biol Med Finland, ISBN 951-98382-1-X, 36-38, 2000). They have numerous biological functions. When mitochondria are activated, caused by bio-immunotherapy, they transform to become electron dense since their cristae gather metallo-enzymes detected as a significant increase in Cr, Fe, Zn, Ti, content, while strontium (Sr) declines. In mammalian cancer cells such transformed mitochondria are involved in biological regulation and repair of the genome (and oncogenes) as cancer cells are forced back into healthy transcription without apoptosis. Organ-specific mitochondria may also specifically activate stem cell maturation (Lancet online 2005, Aug 18, 1-3). They seem to correct mutations as if they have a memory of what they created (Nature 2005: 435,903-910). They may also alter the expression of neuronal genes and mental function in co-operation with billions of vital lipid molecules forming our inductional central nervous system "Lipidomics", acting in concert with Genomics and Proteomics in this triumvirate sustaining normal health. The rare mitochondrial genome mutations are found to be closely linked to lipid metabolism resulting in expression of various forms of neurodegenerative protein and brain-lipid disorders. Mitochondrial mtDNA seems to have a central role in induction and regeneration of the billions of lipids in the CNS, but its short length restricts the transcription capacity, though it is sufficient to induce the 200 organ-specific mitochondria required to regulate all our tissue-specific cells. Organ-specific mitochondria in mammalians can prevent induction of experimental leukemia, cause complete regression of equine sarcoid skin tumours in horses, cure human malignant histiocytoma, melanoma and prostate cancer cells. In electron microscopy they are seen to gather around the dividing nucleus of tumour cells as these regain healthy transcription (Figure). Organ-specific mitochondria may in the future be developed to be utilized as physiological precision medical remedies.



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IDENTIFICATION OF MITOGEN-ACTIVATED PROTEIN KINASE 13 (MAPK13) AS A NOVEL PROGNOSTIC MARKER IN HUMAN CHOLANGIOCARCINOMA

Felicia Tan Li-Sher, Ooi Aik Seng, London Ooi, Tan Yu-Meng, Alexander Chung, Cheow Peng Chung, Soo KC and Teh Bin Tean

Department of General Surgery, Singapore General Hospital;
Department of Surgical Oncology, National Cancer Centre;
NCCS-VARI Translational Cancer Research Laboratory, Singapore

Aim: Cholangiocarcinoma is the second most common type of primary hepatobiliary cancer. It is prevalent in Southeast Asia and worldwide incidence is increasing. Currently, only surgical resection has been shown to improve survival rates. Neither radiation nor chemotherapy significantly improves survival or quality of life. Mechanisms of carcinogenesis remain poorly understood. Prognosis of patients with cholangiocarcinoma is unsatisfactory as there is no reliable tumour marker to facilitate early detection of the disease and no effective chemotherapeutic drugs are available. Our research aims to uncover a biomarker(s) and a prognostic marker(s) for human cholangiocarcinoma using gene expression profiling to enable identification of potential targets for targeted therapeutic therapy. *Methods:* We utilized DNA microarray technology to determine the expression profile in 17 fresh-frozen human cholangiocarcinoma samples. Immunohistochemical staining was then performed on 53 paraffin-embedded cholangiocarcinoma samples. The

data obtained from microarray and IHC was correlated with our clinical data. *Results:* Mitogen-activated protein kinase 13 (MAPK13) overexpression was observed in all our tumour specimens. Subgroup analysis showed that MAPK 13 overexpression correlated with shorter survival time, and survival gradually worsened with increasing MAPK13 scores. MAPK13 overexpression was found to correlate with tumour stage. *Conclusion:* MAPK13 overexpression is a reliable prognostic marker for human cholangiocarcinoma and represents a potential target for targeted therapeutic interventions.

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DEVELOPMENT OF TWO *IN VITRO* ASSAY SYSTEMS: SEPARATION OF CANCER SUBPOPULATIONS AND EVALUATION OF THE MALIGNANT POTENTIALS OF CANCER

Kunihiko Tanaka[1], Yoshihiro Takaya[1], Chiasa Hirakawa[1], Yutaka Tagawa[2,3] and Masami Niwa[1,2]

[1]Department of Pharmacology, Nagasaki University Graduate School of Biomedical Sciences and [2]PharmaCo-Cell Company Limited, 1-12-4 Sakamoto, Nagasaki 852-8523; [3]Department of Nursing, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8520, Japan

To obtain phenotypically distinct subpopulations of cancer cells *in vitro*, we established a new method to separate cancer subpopulations using the Boyden chamber. Two subpopulations of DLD-1 (colon cancer cell line) were separated in which the intercellular junctions differed. Next, we took advantage of the principle and the new assay system (Can kit) which can evaluate the malignant potentials in multistep cancer progression was developed. Can kit was made of two membrane filter chambers, NIH3T3 (mouse fibroblast cell line) and GP8.3 (rat endothelial cell line). HeLa (cervical cancer cell line), B16-F0 (mouse malignant melanoma cell line) and B16-F10 (mouse malignant melanoma cell line) were selected to evaluate Can kit. Each cancer cell line was seeded on Can kit and cultured. After several days, upper chamber was removed and cells dropped in the lower chamber were cultured. MDCK cells (dog kidney epithelial cell line) were seeded on the lower chamber, and transepithelial electrical resistance (TEER) of MDCK was measured after 7 days. HeLa and B16-F10 induced significant reductions of TEER of MDCK, while B16-F0 did not alter it. TEER reductions correlated with the colony areas of dropped cancer cells on the lower chamber. In conclusion, we developed two different *in vitro* assay systems, which are valuable tools to understand the heterogeneity and the malignant potentials of cancer.

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INVOLVEMENT OF THE ESTROGEN RECEPTOR β IN GENISTEIN-INDUCED EXPRESSION OF $P21^{WAF1/CIP1}$ IN PC-3 PROSTATE CANCER CELLS

Tomoaki Tanaka, Kentaro Matsumura, Hidenori Kawashima and Tatsuya Nakatani

Department of Urology, Osaka City University Graduate School of Medicine, Osaka, Japan

Background: Dietary genistein, a phytoestrogen derived from soybean, has been suggested as a chemopreventive agent for prostate cancer. Genistein has been reported to exert its anticancer effects *via* a variety of functional pathways, but the upstream signaling of molecules regulated by genistein remains unclear. In this study, estrogen receptor (ER) β involvement in genistein-induced expression of cell cycle inhibitors in PC-3 prostate cancer cells was investigated. *Materials and Methods:* The proliferation of PC-3 cells exposed to genistein was measured by the water-soluble tetrazolium salt (WST-1) proliferation assay. The expression of p21, p27 and ERβ in the PC-3 cells was assessed by quantitative real-time reverse transcription-PCR. ERβ silencing was performed using a small interfering RNA (siRNA). The transcriptional activity of the p21 promoter was determined by the luciferase reporter assay. *Results:* Genistein caused marked inhibition of proliferative activity and induced the expression of p21 and ERβ in the PC-3 cells. The siRNA against ERβ suppressed the genistein-induced expression of p21 and reduced the transactivation activity of the p21 promoter induced by genistein. *Conclusion:* ERβ is involved in genistein-induced expression of p21 in PC-3 cells.

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COPPER TRANSPORTER ATP7A AND CTR1 EXPRESSIONS IN RELATION TO ACQUIRED RESISTANCE TO CISPLATIN

Toshiko Tanaka[1], Shinobu Matsumoto[2], Hideo Kurokawa[3] and Yutaka Hayashida[4]

[1]Division of Multidisciplinary Studies, Department of Bioscience, [2]Division of Diagnostic Radiology, Department of Oral Diagnostic Science, and [4]Division of Surgery, Department of Control for Physical Functions, Kyushu Dental College, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu 803-8580; [3]Department of Oral and Maxillofacial Surgery, Oita Red Cross Hospital, 3-2-37 Chiyo-machi, Oita 870-0033, Japan

Objectives: Recently, it has been suggested that Cu transporters such as hCTR1, ATP7A, and ATP7B are related to the transfer of CDDP across the cellular membranes, although how CDDP enters and exits tumor cells has

remained poorly understood for years. We investigated whether the expression of CTR1 and ATP7A was related to acquired resistance using CDDP-resistant KBR/0.8 and KBR/1.2 cells, characterized by an impaired intracellular accumulation of CDDP, and CDDP-resistant 2780CP cells, whose mechanisms of CDDP resistance depend on an increased DNA repair ability and not CDDP accumulation. *Methods:* Sensitivity of CDDP was determined using the MTT assay. Intracellular and DNA-bound platinum were measured using atomic absorption spectrophotometry. ATP7A and CTR1 expressions were detected by Western blot analysis. *Results:* KBR/0.8, KBR/1.2, and 2780CP cells were 5.2-, 9.6-, and 4.4-fold more resistant to CDDP compared to parent cell lines, KB, and A2780 cells, respectively. The intracellular CDDP levels in KB, KBR/0.8, and KBR/1.2 cells decreased depending on the degree of resistance after 2-h treatment with 40 and 80 μM CDDP, and those in KBR/1.2 cells were 33% (40 μM) and 27% (80 μM) of that of KB cells. The levels of platinum bound to DNA in KBR/0.8 and KBR/1.2 cells were markedly lower than that of KB cells. The removal of platinum bound to DNA in 2780CP cells was increased by 25.3 and 41.9% at 12 and 24 h after treatment with 40 μM CDDP despite the slight reduction of 15.2% in A2780 cells at 24 h after treatment. The ATP7A level was similar in 2780CP and A2780 cells; however, much lower levels of ATP7A in KBR/1.2 and KBR/0.8 cells were observed compared with KB cells. In cells except for KBR/1.2 and KBR/0.8 cells, ATP expression increased after 2-h exposure to 40 μM CDDP. The level of CTR1 decreased more in 2780CP cells than in A2780 cells. The levels of CTR1 were almost equal among KB, KBR/1.2, and KBR/0/8 cells. Treatment with CDDP hardly had any effect on CTR1 expression in all cell lines. *Conclusion:* ATP7A plays an important role in acquired resistance to CDDP attributed to a decreased intracellular accumulation of CDDP.

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METASTATIC POTENTIAL OF MULTIDRUG-RESISTANT TUMOUR CELLS

Jolanta Tarasiuk and Anna Nowakowska

Department of Biochemistry, University of Szczecin, 3c Felczaka St, 71-412 Szczecin, Poland

Multidrug resistance (MDR) constitutes a major problem in cancer chemotherapy. Tumour cells become resistant to a wide array of chemotherapeutic agents (*e.g.* anthracyclines, *vinca* alkaloids, podophylotoxins, colchicine), structurally diverse and having different mechanisms of action. The occurrence of multidrug resistance (MDR) is conferred by multiple mechanisms, in particular it is associated with the overexpression of membrane transporters (*e.g.* P-glycoprotein, P-gp; MRP1; BCRP/MXR1). These transporters are responsible for the active ATP-dependent efflux of drugs out of resistant cells resulting in reduced intracellular accumulation insufficient to inhibit resistant cell proliferation. Additional multidrug resistance mechanisms of tumour cells are related to: i) drug sequestration into intracellular vesicles, ii) conformational changes of cellular targets (*e.g.* nuclear DNA and topoisomerase II), iii) increase in repair of drug-induced DNA damages, iv) intensification of detoxification processes and v) inhibition of apoptosis. Moreover, in the case of many clinical tumours, it is observed that multidrug-resistant tumour cells have higher invasive and metastatic properties than cells sensitive to chemotherapy. In these cells, the secretion of proangiogenic molecules (*e.g.* vascular endothelial growth factor, VEGF), important changes in cell adhesion molecule expressions and interactions (*e.g.* integrins and E-cadherins) as well as increase in the activity of hydrolytic enzymes responsible for the proteolysis of extracellular matrix proteins such as collagen, laminin and fibronectin (*e.g.* metalloproteinases MMP, cathepsins, enzymes of plasminogen activator system) are often observed.

Identification of mechanisms responsible for MDR phenotype of tumour cells having a high metastatic potential is the main condition in order to develop novel strategies for overcoming multidrug resistance and metastasis of tumour cells. Thus, there are numerous studies focused on the validation of MDR/metastasis targets and identification of new molecular mechanisms responsible for the high metastatic activity of multidrug resistant tumour cells using model MDR metastatic cell lines as well as various clinical drug-resistant metastasis samples (*e.g.* breast, prostate and colorectal carcinomas).

It is proposed that several signal transduction pathways (*e.g.* NF-κB, HIF-1, PI3K/Akt and p53) play a crucial role in the regulation of gene expression involved simultaneously in multidrug resistance and metastasis of tumour cells. This overview addresses recent advances in the understanding of cellular mechanisms responsible for the metastatic potential of multidrug-resistant tumour cells at the gene expression level.

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CONTINUOUS BPH FINASTERIDE THERAPY *VS*. INTERMITTENT TREATMENT: THE UPGRADE OF NED AND THUS OF AGGRESSIVE PROSTATE CANCER

M. Tarle, B. Spajić, I. Kraljić, B. Ruzic, A. Reljic and Z. Kusić

University Hospital Sestre Milosrdnice, Zagreb, Croatia

Introduction and Objectives: Finasteride has been recognized as a drug suitable for the chemoprevention of prostate cancer (PC) by reducing intracellular dihydrotestosterone (DHT) level. Prostate Cancer Prevention Trial (PCPT) data based on

continuous finesteride treatment of almost 19 thousands patients indicated the reduction in cancer prevalence by about 25%. However, in this same study more than a two-fold increase in high grade aggressive prostate tumors was recorded when compared to controls thus arising serious doubts upon the real benefits of the protocol. *Materials and Methods:* During three years a continuous *versus* intermittent (six month treatment followed by 6 months resting period) finasteride treatment in 125 BPH patients (pts) each has been investigated as well as 125 control BPH pts. *Results:* The overall PC prevalence in both finasteride-treated groups was lower than in untreated controls and thus being in accordance with the PCPT data. However, continuous therapy gave significantly higher incidence in Gleason score (GS)>6 carcinomas compared to intermitted therapy and controls (44.5%, 25% and 18.2% of total acquired PC, respectively). In addition, the acquired elevated chromogranin A (CgA) values were also more than doubled in pts treated continuously compared to other two groups (13.6%, 5.6% and 6.4%, respectively). Acquired PC GS>6 recorded in pts with a raise in CgA was higher in continuously treated pts (50%) than in other two studied groups (20% and 25%, respectively). In pts with the retained normal CgA concentration highest PC incidence was found in controls (5.1%) and lower prevalence was recorded in continuously (2.8%) and intermittently treated pts (2.5%) while the respective PC GS>6 incidence was lower in controls than in treated pts. *Conclusion:* Seemingly, finasteride treatment reduces PC prevalence in pts free of NED but elevates the number of aggressive carcinomas in CgA-positive pts only if continuous treatment is applied. In conclusion, current chemoprevention protocols need to be carefully reconsidered prior to the selection between continuous and discontinued finasteride treatment.

The authors are indebted to many colleagues and in particular to Drs. Kraus, Anzulovic, Trnski and Ahel who participated in various stages of the study.

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BLOOD LDH AND CGA VALUES IN HRPC PATIENTS AFTER TEN CYCLES OF CHEMOTHERAPY: MARKERS OF SOFT TISSUE LESIONS AND OSTEOLYSIS

M. Tarle[1], K. Kovacic[1], S. Buhic[2], Z. Kusic[1], I. Kraljic[1] and P. Bouffette[3]

[1]University Hospital Sestre Milosrdnice, Zagreb, Croatia; [2]General Hospital, Dubrovnik, Croatia; [3]American Hospital, Paris, France

Introduction and Objectives: Circulating LDH level was examined as a marker of either soft tissue or bone metastases in prostate cancer (PC) patients (pts) refractory to hormonal control (HRPC pts). *Materials and Methods:* HRPC pts were given chemotherapy (estramustine, docetaxel, corticosteroids, etoposide and related combinations). Serial assessments of clinical, biochemical and hematological data were analyzed either after 8-10 cycles of treatment or maximally eight months. Pts were divided into four groups according to their LDH data (normal, 0-241 U/L) and metastases status.

Elevated LDH level was tested both against pro bnp as a cardiac failure marker and as being an EPO administration side-effect. *Results:* i) 79 pts with bone metastases and no more than 6% increase in LDH level. In 6 months 5 of these pts were switched to group C; ii) 11 pts with bone metastases and elevated LDH value (17-188% increase). Within 6 months lymph node and soft tissue positivity was clinically found in 5/11 (45%) of them. Other 6 pts were followed for possible micrometastases. iii) 14 pts with bone metasteses, soft tissue lesions (lymph nodes, liver/lung), highly elevated LDH (>1000 U/L) and PSA (>500 ng/ml) values were followed. The CrP value was elevated in 10/14 (71%) of these pts. Five pts from group A were added to these 11 subjects due to liver metastases; iv) 8 pts with both bone and soft tissue metastases were found with normal LDH level and an elevated PSA level (92-458 ng/ml); v) In 15/122 (12%) pt with bone lesions osteolysis was recorded and in 13 out of 15 chromogranin A (CgA) serotest level was elevated (>100 ng/ml) together with the elevation of the LDH level. *Conclusion:* According to the ROC curve analysis of the above data both sensitivity and specificity of LDH values in respect to soft tissue lesions are 85-90% thus indicating the interconnection between these two parameters. The simultaneous elevation of CgA and LDH values is indicative of osteolysis. These data open the way for a more detailed analysis of the LDH level in monitoring HRPC pts referred to chemotherapy with the possible extend of the marker to both osteolysis and soft tissue micrometastases.

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CHROMOSOME COPY-NUMBER VARIATION AND BREAST CANCER RISK

Sandrine Tchatchou and Barbara Burwinkel

Helmholtz-University Group Molecular Epidemiology, German Cancer Research Center, DKFZ, Heidelberg, Germany

Breast cancer is the most frequent cancer among women. It is caused by genetic and environmental factors. Whereas mutations in high-penetrance susceptibility genes have been identified in familial breast cancer and several single nucleotide polymorphisms (SNPs) have been shown to be associated with both familial and sporadic breast cancer risk, the impact of genomic copy-number variants (CNVs) on breast cancer risk has so far poorly been studied. An example of a CNV affecting the tumor suppressor gene *MTUS1* that has been to be

associated with familial breast cancer risk is given. Moreover, we discuss CNVs affecting detoxification genes such as *GSTM1* and *GSTT1* that were controversially associated with breast cancer risk. Finally, the potential of array-based genome-wide CNV association studies is discussed.

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EXTRACELLULAR PROTEASES ACTIVITY IN A TUMOR PROGRESSION EXPERIMENTAL MODEL

E.M. Tchevkina, A.V. Knizhnik, V.A. Rybko, Y.A. Kainov and A.V. Komelkov

Department of Oncogene Regulation, Carcinogenesis Research Institute, N.N. Blokhin Memorial Cancer Research Center, Moscow, Russia

The study was focused on comparative analysis of extracellular proteases activity *in vitro* in high and low metastatic lines of transformed hamster fibroblasts, as well as *in vivo* in primary tumors and metastatic lung samples of experimental animals followed by subcutaneous injection of studied cell lines. We compared extracellular proteases activity in highly tumorigenic cell lines of the same origin (RSV transformed hamster embryo fibroblasts) with different levels of lung metastatic activity. We also analysed this characteristic in primary tumors and lungs of hamsters after subcutaneous injection of studied cells. Activity of extracellular proteases, such as urokinase type plasminogen activator (uPA) and matrix metalloproteinases MMP-1, -2 and -9, was determined using casein-plasminogen and gelatinase zymography in cell culture media, cell lysates, lysates of tumour samples and lung metastatic lesions, respectively. uPA receptor (uPAR) expression was detected by Western-blot analysis.

We showed that uPA activity (both intracellular and secreted) is significantly elevated in high metastatic HET-SR1 line in comparison with low metastatic HET-SR line. An increase of uPA activity was also found in primary tumours as well as in the samples of metastatic lungs of animals after injection of HET-SR1 cells in comparison with hamsters injected with HET-SR cells. Both lines demonstrated similar high level of uPAR protein expression. MMP-1, -2 and -9 activity analysis revealed that *in vitro* in culltured cells, MMP-2 is the most active secreted gelatinase, MMP-1 is less active and MMP-9 activity is practically undetectable. *In vivo*, MMP-9 activity is stimulated so that in tumor samples MMP-9 and MMP-2 demonstrate comparable levels of activity. MMP-9 is the most active gelatinase in lungs. Under the conditions of carcinogenesis and metastasis, MMP-9 activity in lung cancer increases in comparison with that of healthy animals from control group. MMP-2 secretion *in vitro* is elevated in the high metastatic HET-SR1 cell line in comparison with HET-SR cells. *In vivo*, MMP-9 and MMP-2 are more active both in primary tumors and in lung samples of hamsters injected with HET-SR1 cells.

uPA, MMP-2 and MMP-9 activities are critical for the development of highly metastatic cell phenotype in the studied experimental model.

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APE1/REF-1: A DNA REPAIR PROTEIN WITH PLEIOTROPIC ACTIONS

Gianluca Tell

Molecular Biology Section, School of Medicine, Department of Biomedical Sciences and Technologies, University of Udine, P.le Kolbe 4, 33100 Udine, Italy

APE1/Ref-1 (APE1), the mammalian ortholog of E. coli Xth, and a multifunctional protein possessing both DNA repair and transcriptional regulatory activities, has pleiotropic role in controlling cellular response to oxidative stress. APE1 is a vital protein. It is the main apurinic/apyrimidinic endonuclease in eukaryotic cells playing a central role in the DNA base excision repair pathway of all DNA lesions (uracil, alkylated and oxidized and abasic sites), including single-strand breaks, and has also co-transcriptional activity by modulating gene expression directly regulated by either ubiquitous (*i.e.* AP-1, Egr-1, NF-κB, p53, HIF) or tissue-specific (*i.e.* PEBP-2, Pax-5 and -8, TTF-1) transcription factors. Thus it plays a central role in controlling different, and apparently contrasting, cellular processes such as apoptosis, proliferation and differentiation. It may also have quite diversified roles depending on the cell type, whether normally dividing, postmitotic, or cancer cells. In addition, it controls the intracellular redox state by inhibiting reactive oxygen species (ROS) production. We speculate that the essentiality of APE1 may be due to its pleiotropic biological effects rather than merely to its DNA-repair activity. To this aim, the biological relevance of APE1 in eukaryotic transcriptional regulation of gene expression has yet to be elucidated, but progress is being made using inhibitors of specific APE1 functions. The ability of APE1 to activate transcription factors, such as p53 and Egr-1, which are mainly involved in controlling cell-cycle-arrest and apoptotic programs, leaves the debate open about the mechanisms responsible for controlling its different functions in several contexts. At present, information is still inadequate regarding the molecular mechanisms responsible for the coordinated control of its several activities. However, change in the expression level, subcellular distribution, post-translational modifications and modulation of its interacting partners may significantly contribute to the fine-tuning of its different activities. Both expression and/or subcellular localization are altered in several metabolic and proliferative disorders such as in tumors and aging. In the present review, we have attempted to relate the most relevant informations, including our most recent findings on the APE1 interactome and

gene networking concerning different functions of APE1 in order to shed new light and to focus current and future studies to fully understand this unique molecule that is acquiring more and more interest and translational relevance in the field of molecular cancer therapeutics.

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UV BIODOSIMETER FOR ANTICANCER UV EXPOSURE – VISUAL DETECTION OF VITAMIN D SYNTHESIS

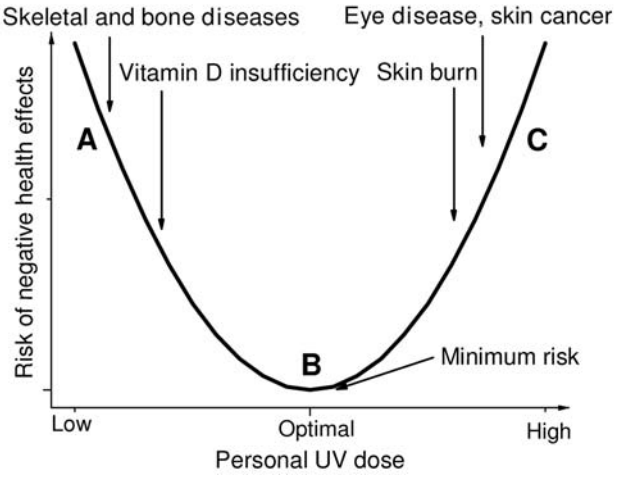
Irina Terenetskaya

Institute of Physics, National Academy of Sciences of Ukraine, 02028, Kiev, Ukraine

As is well known, high energy ultraviolet (UV) photons may produce positive or negative health effects depending on the received UV dose. Excessive UV exposures are generally associated with acute and chronic effects, and most commonly used UV detectors have spectral sensitivity which is a close match to the CIE erythema action spectrum.

Nevertheless, in proper dose UV radiation is beneficial: in addition to psychological benefits, exposure to UV-B light (280-315 nm) helps the body to produce vitamin D, which is recognized as a critical factor of cells' growth. Epidemiological studies show that cancer mortality rates are correlated inversely with local solar UV-B doses for 13 types of cancer, and the most likely mechanism whereby solar UV-B radiation provides protection against cancer is natural production of vitamin D.

In the meantime, the vitamin D deficiency has become a neglected epidemic in most adults who are not exposed to adequate sunlight. With due regard to recent data on the Europe's darker atmosphere in the UV-B, it is believed vitamin D deficiency can be treated by artificial irradiation on condition that the vitamin D synthetic capacity is properly detected to avoid both either UV deprivation or acute and chronic UV injury (Figure).



To determine quantitatively the boundaries near area B, the so-called "comfort UV dose", we have developed a bio-equivalent UV dosimeter1) that is based on the same molecular photochemistry from which vitamin D is synthesized in human skin, *i.e.* 7-dehydrocholesterol (7-DHC, provitamin D3) photoconversion. Furthermore, to visualize previtamin D photosynthesis, 7-DHC was dissolved in liquid crystalline matrix. Under UV irradiation the molecular conformation of 7-DHC molecule is altered, and the LC sample changes its colour. This enables quantitative estimation of previtamin D synthesized per cm^2 using calibrated color strips (like a litmus paper measures pH) providing the easiest detection of vitamin D synthesis and simple estimation *in situ* of the accepted "antirachitic" UV dose.

1 Orlova T and Terenetskaya I: "UV-biosensor for visual indication of vitamin D synthesis". SPIE "Optical Sensors 2008", v.7003, (70031O-1).

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PROSPECTIVE EVALUATION OF SEXUAL FUNCTION AFTER "OPEN" AND LAPAROSCOPIC SURGERY FOR RECTAL CANCER

Paraskevas Stamopoulos[1], George E. Theodoropoulos[1], Joanna Papailiou[1], Dimitris Savidis[2], Christina Golemati[1], Konstandinos Bramis[3], Sotirios-George Panoussopoulos[1] and Emmanouil Leandros[1]

[1]First Department of Propaideutic Surgery of Athens Medical School, Hippocration University Hospital, 114 Vas Sofias Ave, 11527, Athens;
[2]Department of Urology, Hippocration University Hospital, 114 Vas Sofias Ave, 11527, Athens;
[3]First Department of Surgery of Athens Medical School, Laikon University Hospital, 17 Ag. Thoma Str, 11527, Athens, Greece

Background: Sexual function may be harmed after treatment for rectal cancer. Aim of this study is to prospectively evaluate the incidence of sexual dysfunction after rectal cancer treatment and to compare the effects of laparoscopic and traditional "open" approaches in postoperative sexual function. *Methods:* Baseline, 3, 6 and 12 month assessments of sexual dysfunction using the International Index of Erectile Function (IIEF) and its specific domains prospectively took place in 56 patients who underwent rectal cancer surgery (38 "open" procedures, 38 low anterior resections). The preliminary results are presented. *Results:* The average total IIEF and isolated IIEF response domain scores were significantly decreased after surgery ($p<0.01$), except for "intercourse satisfaction" and "overall satisfaction" scores at 12 months. An improvement of IIEF scores was observed between the 3- and 6-month assessment

points (p<0.01), except for the "erectile function" and "orgasmic function" scores. No significant differences were observed between the "open" and laparoscopic group when comparing total IIEF and domain scores at preoperative, 3- and 6-month assessment points. Rates of sexual dysfunction did not differ significantly preoperatively and at 3 months postoperatively when "open" and laparoscopic procedures were compared, although there was a trend in favour of laparoscopic surgery at 6 months (p=0.076). Baseline IIEF score and baseline, 3- and 6-month "sexual desire" scores were better (p=0.035, p=0.004, p=0.017 and p=0.061, respectively) between the low anterior resection *vs*. the abdominoperineal resection groups. *Conclusion:* Rectal cancer resections were postoperatively associated with significant reduction of IIEF scores and high rates of sexual dysfunction at 3 and 6 months. IIEF and domain scores at different assessment points were comparable between the laparoscopic and "open" surgery groups. Extending the monitoring period and adding more patients in this ongoing prospective study will further elucidate the postoperative sexual dysfunction after rectal cancer surgery.

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PROSPECTIVE EVALUATION OF HEALTH-RELATED QUALITY OF LIFE IN A SOLID MEDITERRANEAN GROUP OF COLORECTAL CANCER PATIENTS

George E. Theodoropoulos[1], Joanna Papailiou[1], Paraskevas Stamopoulos[1], Christina Golemati[1], Konstandinos Bramis[2], Sotirios-George Panoussopoulos[1] and Emmanouil Leandros[1]

[1]First Department of Propaideutic Surgery of Athens Medical School, Hippocration University Hospital, 114 Vas Sofias Ave, 11527, Athens;
[2]First Department of Surgery of Athens Medical School, Laikon University Hospital 17 Ag. Thoma Str, 11527, Athens, Greece

Background: The primary aims of colorectal cancer surgery are to achieve oncological clearance and to prolong survival. Health-Related Quality of Life must not be forgotten in the quest for oncological excellence. Ethnic background affects the patients' perception of quality of life. This study was designed to prospectively evaluate Health Related Quality of Life, in a solid Mediterranean group of colorectal cancer patients. *Methods:* Ninety-five colorectal cancer patients were preoperatively assessed and followed up in repetitive postoperative fixed time-points. The Short Form-36 Health Survey questionnaire was used by skilled investigators. *Results:* Overall, patients showed deterioration in all domains, except for pain, when baseline values were compared to 3 and 6 months postoperatively (p=0.0001). A significant improvement of SF-36 domains was noted between 6 and 12 months (p=0.0001). Scores for general health, pain, emotional wellbeing, role limitations due to emotional problems at 1 year were better than preoperative (p<0.001). Better role limitations due to physical health and due to emotional problems domains scores at baseline and at 1 year were found when laparoscopic were compared to open resections (p<0.05). Patients that received chemotherapy, proved to be more susceptible regarding their energy, social functioning and role limitations at 3 months (p<0.05). Older patients had diminished physical functioning at 3 and 6 and 12 months (p<0.05). *Conclusion:* Colorectal cancer patients remain fragile up to 6 months postoperatively, with significant improvements at 1 year. Certain aspects of health related quality of life at 1 year may be even better than before surgery.

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p53 AND EGFR EXPRESSION IN COLORECTAL CANCER: A REAPPRAISAL OF "OLD" TISSUE MARKERS IN PATIENTS WITH LONG FOLLOW-UP

George E. Theodoropoulos[1], Eleni Karafoka[2], Joanna Papailiou[1], Paraskevas Stamopoulos[1], Konstantinos Zaibirinis[1], Konstandinos Bramis[3], Sotirios-George Panoussopoulos[1] and John Bramis[1]

[1]First Department of Propaideutic Surgery of Athens Medical School, Hippocration University Hospital, 114 Vas Sofias Ave, 11527, Athens;
[2]Lefkos Stavros Hospital,1 Sisini Str, 11528, Athens;
[3]First Department of Surgery of Athens Medical School, Laikon University Hospital 17 Ag. Thoma Str, 11527, Athens, Greece

Background: Extensive research into the biology of colorectal cancer has identified a plethora of molecular markers reputed to provide prognostic information. During the last two decades conflicting results have been drawn on the role of the p53 tumor suppressor gene and of the first identified member of the type receptor tyrosine kinase family, the EGFR on colorectal cancer prognosis. p53 mutational status has been associated with both improved and reduced survival. EGFR has been associated with decreased length of survival, increasing Dukes stage or lymph node metastases in several reports, but as many studies have reported no association with unfavourable prognostic parameters. The aim of the study was to evaluate the p53 and EGFR expression in patients with an at least 5-year follow-up. *Materials and Methods:* Paraffin-embedded material was retrospectively collected from 164 colorectal adenocarinoma (50 rectal cancers) patients, who had been operated between 1994 and 2003. Median follow-up was 5 years (range: 1-14). p53 and EGFR expression was evaluated

by immunohistochemistry. *Results:* Positive p53 immunostaining and EGFR expression was observed at 62% and 43%, respectively. P53 and EGFR positivity rates were significantly interrelated (p=0.004). No significant correlation was found with the examined clinicopathological parameters except for the advanced T-stage, which demonstrated significant associations with the p53 expression (p=0.004), the EGFR expression (p=0.0001) and the p53/EGFR co-expression (p=0.001). In univariate survival analysis (Log rank test) the stage (p=0.0001), the lymphovascular invasion (p=0.005), the perineural infiltration (p=0.004) were associated with the overall cancer-specific survival, while a trend existed for EGFR (p=0.06) and p53/EGFR co-expression (p=0.07). At multivariate analysis only stage was associated with increased risk of cancer death (Cox regression analysis p=0.0001, b coefficient (SE): 1.898 (0.383). *Conclusion:* P53 and EGFR were overexpressed in this colorectal cancer patient population and were significantly associated with advanced T stage. In the context of new therapeutic strategies using EGFR-targeted therapies, although EGFR remains a controversial prognostic factor, this expression–stage association may play a crucial role in a decision to initiate an adjuvant treatment.

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HEALTH-RELATED QUALITY OF LIFE ISSUES AFTER LAPAROSCOPIC AND OPEN RESECTIONS FOR COLORECTAL CANCER: THE INTERNATIONAL PERSPECTIVES AND THE EXPERIENCE FROM A GREEK UNIVERSITY HOSPITAL

George E. Theodoropoulos

Department of Colon and Rectal Surgery, Athens Medical School, Greece

Colorectal cancer (CRC) is one of the most common malignancies in the Western part of the world. Surgery is the mainstay of treatment, and it may be combined with preoperative radiotherapy or adjuvant chemotherapy. Although surgery offers a good chance of cure, it also has short and long-term detrimental impacts on patients' health-related quality of life (HRQL). For several post-operative months, patients experience fatigue, pain and reduced activity levels. Many struggle with an altered bowel habit, and the patient may need to adapt to life with a permanent or temporary stoma. After rectal surgery, sexual and urinary dysfunction frequently occurs, and these problems may persist. The diagnosis and treatment of CRC also has a psychosocial impact; patients worry about disease recurrence, and the combination of physical and emotional difficulties inevitably impact on social well-being.

Laparoscopic colorectal surgery for cancer offers short-term advantages such as earlier diet re-establishment, less postoperative pain, less narcotic use and shorter hospital stay. Slowly accumulating and rather controversial is the information in regards to the effect of laparoscopic colorectal resections on HRQL. Four randomized controlled trials exist in the literature comparing "open" and laparoscopic colectomies for cancer. Analyzing the early results of the US COST study, Weeks et al. reported that laparoscopic surgery resulted in slightly better overall HRQL 2 weeks post-procedure and less pain. The small trial by King et al. reported no HRQL differences between treatment groups as did the larger UK multi-centre trial (CLASSIC). CLASSIC study is probably the most informative, as it contains information on HRQL derived from repetitive assessments at fixed intervals. In this study, more problems than at baseline were reported for physical functioning by both "open" and laparoscopic arms at 6 months postoperatively. This continued in the laparoscopic arm up to 3 years, but returned to baseline levels at 18 months for the open arm. Fewer problems than at baseline were reported by both arms for emotional functioning. Social functioning was worse than at baseline for the laparoscopic arm up to 3 years postoperatively, but remained the same as baseline for the open arm. There was more fatigue for both arms at 6 months but levels returned to baseline for both arms by 18 months. In a small study, Schwenk *et al*. reported significantly less pain and fatigue after laparoscopic colon resections.

Eight studies in the international literature report data on sexual dysfunction after laparoscopic total mesorectal excision (TME) for rectal cancer.A retrospective, questionnaire-based study by Quah *et al*. showed a higher rate of male sexual dysfunction after laparoscopic resection compared with open resection Analyzing the prospectively collected data of the CLASSIC study, Jayne *et al*. found that overall sexual function tended to be worse after laparoscopic than "open" rectal surgery. A recent Turkish study between the two approaches, though, demonstrated that impotency rates were higher after "open" surgery. Focusing on patients with low-lying rectal cancers undergoing sphincter-preserving operations, Yang *et al*. demonstrated that laparoscopic TME male patients had better sexual function and less sexual problems during 12-18 months and better sexual enjoyment than "open" TME patients. In a small series recently published by Breukink *et al*., erectile function was maintained in 71% and ejaculation function in 89%, while a significant deterioration in intercourse satisfaction with unchanged the overall satisfaction was observed after radiotherapy and laparoscopic TME.

In our Institution, 95 colorectal cancer patients were preoperatively assessed and followed up in repetitive postoperative fixed time-points. The Short Form-36 Health Survey questionnaire was used for HRQL assessment.

Colorectal cancer patients remain fragile up to 6 months postoperatively, with significant improvement at 1 year. Certain aspects of health related quality of life at 1 year may be even better than before surgery. Better role limitations due to physical health and due to emotional problems domains scores at baseline and at 1 year were found when laparoscopic were compared to open resections. In 56 patients who underwent rectal cancer surgery (38 "open" procedures, 18 laparoscopic), a significant reduction of International Index for Erectile Function (IIEF) scores and high rates of sexual dysfunction at 3 and 6 months were observed. IIEF and domain scores at different assessment points were comparable between the laparoscopic and "open" surgery groups

Although the primary aims of colorectal cancer surgery are to achieve oncological clearance and to prolong survival. Health-Related Quality of Life must not be forgotten in the quest for oncological excellence.

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EXPRESSION OF PROSTAGLANDIN METABOLIZING ENZYMES IN CORRELATION WITH VITAMIN D RECEPTOR IN BENIGN AND MALIGNANT BREAST CELL LINES AND BREAST TISSUE

M. Thill[1], D. Fischer[1], S. Becker[1], T. Cordes[1], K. Diedrich[1] and M. Friedrich[2]

1Department of Obstetrics and Gynecology, University Schleswig-Holstein, Campus Luebeck, Ratzeburger Allee 160, 23538 Luebeck;
2Department of Gynecology and Obstetrics, Helios Hospital Krefeld, Lutherplatz 40, 47805 Krefeld, Germany

Background: The antiproliferative effects of calcitriol [1,25$(OH)_2D_3$] make the biologically active form of vitamin D to a promising target in breast cancer therapy. It is well known, that these effects are mediated *via* the vitamin D_3 receptor (VDR). Furthermore breast cancer is associated with inflammatory processes based on an up-regulation of cyclooxygenase-2 (COX-2) expression, the prostaglandin E_2 (PGE2) synthesizing enzyme. The PGE_2 metabolizing enzyme, 15 hydroxyprostaglandin dehydrogenase (15PGDH) is described as a tumor suppressor in cancer. First references suggest a correlation between vitamin D and prostaglandin metabolism through the impact of 1,25$(OH)_2D_3$ on the expression of COX-2 and 15PGDH. Thus we evaluated the expression of COX-2 and 15PGDH in correlation with VDR. *Materials and Methods:* The Expression of VDR, COX-2 and 15PGDH in MCF10F, a human benign epithelial cell line and the breast cancer cell line, MCF-7 was determined by real-time PCR and western blot analysis. Furthermore, we determined mRNA levels of COX-2 and 15PGDH and VDR in healthy and malignant breast sample tissues. *Results:* Although our data from real-time PCR were divergent from those obtained from the Western-blot analysis, COX-2 protein expression increased in MCF-7 2-fold compared to MCF10F. These data were confirmed in breast cancer tissue samples, where COX-2 mRNA level increased by 1.44 in comparison to healthy tissues. No correlation of 15PGDH was detected in either cell line, but in malignant tissues, 15PGDH increase dramatically compared to healthy tissue samples. The expression of VDR differed at the protein and mRNA level in benign and malignant cell lines. VDR protein levels were inversely correlated to 15PGDH expression and revealed that the benign tissues as well as the MCF10F cells have the highest VDR expression. *Conclusion:* We found a basically inverse correlation between VDR and 15PGDH protein level expression. These findings suggest a possible link between VDR, associated target genes and prostaglandin metabolism. Gaining further insight into mechanisms regulating VDR, COX-2 and 15PGDH expression in healthy and malign tissue might open new therapeutic approaches in breast cancer therapy.

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ASSOCIATION BETWEEN *XRCC 1* POLYMORPHISMS AND HEAD AND NECK CANCER IN HUNGARIAN POPULATION

Andras Csejtei[1], Antal Tibold[2], Katalin Koltai[3], Zsuzsa Varga[4], Istvan Szanyi[5], Gyula Gobel[5], Ida Prantner[2], Denes Steffler[2], Istvan Ember[2] and Istvan Kiss[2]

[1]Department of Oncoradiology, Markusovszky Hospital, Hungary, Szombathely, Markusovszky Lajos str. 3;
[2]Department of Public Health, University of Pécs, Medical School, Hungary, Pécs, Szigeti str. 12;
[3]1st Department of Medicine, University of Pécs, Medical School, Hungary, Pécs, Ifjúság str. 13;
[4]Department of Oncology, Baranya County Hospital, Hungary, Pécs, Rákóczi str. 2;
[5]Department of Oto-rhino-laryngology, University of Pécs, Medical School, Hungary, Pécs, Munkácsy M. str. 2, Hungary

Background: The head and neck cancer is the fifth most common newly diagnosed cancer in the Hungarian population, with a mortality that increased by 265% in the last thirty years. Tobacco use and alcohol consumption are the most important risk factors of head and neck cancer. Because of the important role of the XRCC1 gene in DNA repair, we tested the effects of the *Arg194Trp* and *Arg399Gln* polymorphisms of XRCC1 to the clinical outcome of the head and neck cancer. *Materials and Methods:* A PCR-RFLP method was used. 108 samples were taken from intraoperatively removed formalin fixed,

and paraffin embedded blocks of tissue. After deparaffination by microwave extraction, samples were digested with proteinase-K. For PCR amplification the following primers were used: 5'-GCC AGG GCC CCT CCT TCA A-3' and 5'-TAC CCT CAG ACC CAC GAG T-3' for *Arg194Trp* polymorphism and 5'-TGC TTT CTC TGT GTC CA-3' and 5'-TCC AGC CTT TTC TGA TA-3' for *Arg399Gln* polymorphism. The restriction enyzyme *PvuII* was used to distinguish the *Arg194Trp* polymorphism and *MspI* enzyme to distinguish the *Arg399Gln* polymorphism. An age-and sex-matched healthy control group was used to compare the frequency of polymorphic variants with cancer-free population. *Results and Discussion:* No significant difference was found between patients and controls regarding the investigated polymorphisms of *XRCC1* gene. In the second part of our study we tested the effects of genotype effects on overall survival. We investigated the effects of the *XRCC1* gene polymorphisms in respect to the patients clinical stage. We found a significant difference between patients with different *XRCC1* 194 polymorph status in clinical stage SIII. The patients with *Arg194Arg* genotype had significantly lower survival rate than patients with *Arg194Trp* genotype. The complex analysis of these factors may be a way for the personal risk assesement and treatment.

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ALLELIC POLYMORPHISMS AS MODIFILERS OF CLINICAL OUTCOME IN COLORECTAL CANCER

Antal Tibold[1], Andras Csejtei[2], Zsuzsa Varga[3], Katalin Koltai[4], Agoston Ember[5], Zsuzsa Orsos[1], Istvan Ember[1] and Istvan Kiss[1]

[1]Department of Public Health, University of Pécs, Medical School, Hungary, Pécs, Szigeti str. 12;
[2]Department of Oncoradiology, Markusovszky Hospital, Hungary, Szombathely, Markusovszky Lajos str. 3;
[3]Department of Oncology, Baranya County Hospital, Hungary, Pécs, Rákóczi str. 2;
[4]1st Department of Medicine, University of Pécs, Medical School, Hungary, Pécs, Ifjúság str. 13;
[5]Department of Surgery, University of Pécs, Medical School, Pécs, Ifjúság str. 13, Hungary

Background: Cancers of the colorectal region are the second most frequent cause of death among malignant diseases. We investigated the influence of two allelic polymorphisms of the GSTM1 and GSTT1 metabolyzing enzymes and that of p53 tumor suppressor gene codon 72 polymorphisms on colon cancer. *Materials and Methods:* 102 intraoperatively removed tissue samples from patients with colorectal cancer were processed. Cancer–free human samples were used as matched controls. After deparaffination, samples were digested with proteinase-K. DNA solutions were used for PCR amplification. For amplification the following primers were used: GSTT1 forward primer 5'-TT CCT TAC TGG TCC TCA CAT CTC-3'; GSTT1, reverse primer 5'-TCA CCG GAT CAT GGC CAG CA -3'; and GST M1 forward primer: 5'-GAA CTC CCT GAA AAG CTA AAG C-3', GSTM1 reverse primer 5'-GTT GGG CTC AAA TAT ACG GTG G-3'. P53 genotyping (codon 72, Arg/Pro polymorphism) was performed using 3' primer: GC AAC TGA CCG TGC AAG TCA and 5' primers for Arg variant ATGCCAGAGGCTGCTCCCCG and for the Pro allele ATG CCA GAG GCT GCT CCC CC. *Results and Discussion:* No significant difference was found between cancer patients and controls in respect to GSTM1, GSTT1 and p53 polymorphisms. Kaplan–Meier curves were defined in all Dukes' stages. Significant association was found in stage Dukes' B patients between the GSTM1 and p53 gene variant and survival. The chance of survival was significantly lower in patients with GSTM1 0 genotype and in p53 Arg/Pro heterozygotes or Pro/Pro homozygotes than in the case of GSTM1+ and p53 Arg/Arg variants (p=0.0089 and p=0.0008). The relevance of the investigated polymorphisms on prognosis is dependent on the tumor stage. These parameters might be used in certain cases as prognostic biomarkers and in the planning of individualized therapy.

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METASTATIC GENE SIGNATURE OF THE MOST AGGRESSIVE HUMAN CANCER: CUTANEOUS MELANOMA

J. Tímár[1,2], L. Mészáros[1], A. Ladányi[1], L. Puskás[3] and E. Rásó[1]

[1]National Institute of Oncology, Budapest;
[2]Department of Pathology, Semmelweis University, Budapest;
[3]Center of Biology, Hungarian Academy of Sciences, Szeged, Hungary

Most of the melanoma markers used today are melanocytic markers or pigmentation pathway-associated genes driven by the microphthalmia transcription factor, MITF, and include, among others, tyrosinase, dopachrome tautomerase, DCT, melan-A and S100B. Genomic studies repeatedly revealed several novel melanoma marker genes including those of the transcription factor NOTCH2, WNT5A, proliferation-associated genes *TOPO2A* and *CDC2*, membrane receptors FGFR and EphA3, adhesion molecules N-cadherin, β3 integrin and syndecan-4, and the cell surface antigens CD59/protectin and MIA. Other genomic analyses tried to define the gene signature of the metastatic disease but failed to find a consistent one except

the gold standard genes of *β3 integrin, syndecan-4* and *WNT5a*. Studies on the gene signatures of chemoresistance and cytokine sensitivity of melanoma clearly defined apoptosis-resistance as one of the key elements of the above biological properties, but the data are controversial, mostly because of the use of inappropriate model systems and the lack of confirmation on clinical samples. Recently a meta-analysis of the published array data (involving eight hundred genes) identified only a few-gene set of *IGFBP, CMET, FGFR1* and *CDK2* which repeatedly (at least in 3 cases) occured in the various genomic studies on metastatic melanoma suggesting that without proper validation of the gene sets and proper selection of the histological variants of melanoma it would not be feasible to define a better prognostic signature.

We have used three genetically unrelated human melanoma cell lines grown as *s.c.* xenografts in SCID mice to reveal a metastatic melanoma gene signature in the primary tumor. To identify relevant genes, we used newborn host (metastatic condition) and adult host (nonmetastatic condition). Since the stroma is murine, it is possible to separate the human (melanoma) genes from the stromal genes without microdissection. Expression profiles of primary human melanomas were obtained by the 41k Agilent Whole Human Genome Oligo Microarray. The gene set significantly different in all the three cell lines was further validated by qPCR using Taqman card of 96 genes (Applied Biosystem). Using this model, we have identified a large 832 metastatic melanoma gene signature ($p<0.05$), from which we were able to derive a 39-gene set validated by qPCR. This melanoma metastasis gene signature contained 9 previously reported metastasis genes including *transmembrane heparan sulphate proteoglycan, β3 integrin, AKT* and *TOPO2A*. The largest group of metastasis genes belongs to those involved in the regulation of apoptosis (8) and those involved in developmental regulation (*NOTCH1, FOXD3, FZD9, HOXC13*). This 39-gene set was validated on a clinical cohort of twenty primary SSM melanoma samples where the 5-year history of clinical behaviour was known (visceral metastatic progression or locoregional dissemination/no progression). Using the TaqMan technology we were able to identify a 17-gene metastatic melanoma gene signature of ten up-regulated and 7 down-regulated genes. The signature contains *integrin* a_v*, RXRγ, TOPO2A, NOTHCH1* and *IGFBP7,* all claimed previously to be involved in melanoma progression. The signature also contains a 4-gene set, members of which are involved in apoptosis regulation. This novel metastastatic melanoma gene signature revealed the apoptotic pathway, TOPO2A and β3 integrin as valid molecular therapeutic targets of melanoma progression. This study was supported by NKFP1a-0024-05.

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A ROLE FOR NON-CODING RNA IN CANCER EPIGENETICS

Angela H. Ting

Cleveland Clinic, Ohio, USA

Epigenetic abnormalities, which involve modifications to the DNA and histones, have long been recognized for their pivotal role in silencing protein coding genes (tumor suppressors) to allow cancer development. The involvement of non-coding RNAs will be discussed in the context of aberrant epigenetic silencing in cancer and data on RNA-dependent transcriptional silencing as a potential mechanism for the initiation/maintenance of cancer epigenetics will be presented. We found that synthetic double-stranded RNA is able to effectively initiate transcriptional gene silencing in human cancer cell lines. Furthermore, loss of DICER, an essential non-coding RNA processing enzyme, results in the loss of localized promoter hypermethylation and gene silencing. Together, these data support our hypothesis that an RNA-mediated pathway is responsible for at least some of the epigenetic regulation in human cancer.

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EXPRESSION OF ANGIOPOIETINS AND TIE-2 RECEPTOR IN GASTROINTESTINAL CARCINOMAS

Paola Tonino[1], Elizabeth Valdivieso[2] and Carmen Abreu[3]

[1]Centro de Microscopía Electrónica "Dr. Mitsuo Ogura", Facultad de Ciencias, Universidad Central de Venezuela, Apartado 76963, El Marques 1070, Caracas, Venezuela; [2]Instituto de Biología Experimental, Facultad de Ciencias, [3]Instituto Anatomopatológico, Facultad de Medicina, Universidad Central de Venezuela

The angiopoietins 1-4 (Ang1 – Ang4) are a family of growth factors identified as ligands for Tie2 receptor tyrosine kinase, critically involved in angiogenesis by their regulation of endothelial survival. Ang1 acts as an agonist, activating the Tie2 signaling pathways, whereas Ang2 acts as an antagonist, specifically blocking the Ang1 dependent activation in endothelial cells. Ang3 (mouse) and Ang4 (human) also show context-dependent actions as antagonistic and agonistic ligands, respectively. The biological actions of the Angs in tumor growth and metastasis in gastrointestinal cancer are still not fully understood. The aim of the present study was to evaluate the expression of Ang1, Ang2, Ang4 and Tie2 receptor in human gastrointestinal carcinomas (GITC), and their relation to clinicopathological factors. Immunohistochemical and Western blot analysis were performed in surgically resected specimens from patients

(n=27). Normal gastrointestinal mucosa was examined as normal controls (n=5). Intensity of immunostaining was scored as negative, weak, moderate and high. Mann-Whitney and Chi-square test were used for statistical comparisons with a significance defined as $p<0.05$. Normal gastrointestinal epithelium showed a negative or weak immunoreactivity for Ang1, Ang2, Ang4 and Tie2 receptor. Of the GITC, 77.78% (21/27), 74.07% (20/27), 85.19% (23/27) and 59.26% cases (16/27) showed positive immunostaining in the cytoplasm and nucleus of tumor cells, and endothelial cells for Ang1, Ang2, Ang4 and Tie2, respectively. A moderate Ang4 > Ang2 > Ang1 immunoreactions was seen in most GITC, however the higher intensity of immunostaining was observed as Ang1 > Ang2 > Ang4 when compared to non-malignant epithelial cells. The expression of Ang1, Ang2, Ang4 and Tie2 was significantly correlated to the venous invasion ($p<0.01$, $p<0.001$, $p<0.05$ and $p<0.001$, respectively) and lymph node metastasis ($p<0.001$, $p<0.01$, $p<0.01$ and $p<0.001$, respectively). Western blot analysis (n=13) from GITC revealed an upregulation of Ang1 (92.30%; 12/13), Ang2 (84.61%, 11/13), Ang4 (92.30%, 12/13), and Tie2 receptor (76.92%, 10/13) in correspondence with the immunohistochemical expression. The overexpression of Ang1, Ang2, Ang4 and Tie2 in human GITC suggests that signaling pathways *via* Ang-Tie2 ligand-receptor is involved in the invasion and metastatic potential of these tumors, and certainly highlights that these factors represent important therapeutic targets to regulate angiogenesis.

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THE BIOLOGICAL ACTIVITY OF TUMOR IN ROUTINE PRACTICE

O. Topolčan, L. Holubec, V. Třeška, M. Pešek and J. Fínek

Faculty Hospital and Medical School in Pilsen, E. Benese 13, 305 99 Plzen, Czech Republic

Aim: The aim of the study is to demonstrate a potential clinical use of tumor marker assessment. A current indication for the tumor markers assessment for the primary diagnosis, follow-up and therapy monitoring is based on ROC of individual markers, obtained from multicenter study results. This study proposes an opposite approach: to estimate clinician's needs and to propose the optimal combination of tumor markers. *Results and Discussion:* On the basis of the evaluation of results from more than 300,000 routine analyses, an optimal multiparametric diagnostic procedure for follow up of 10 most common tumor diseases has been elaborated. The possibility of its use in diagnostics using multiplex diagnostic methods is discussed. *Conclusion:* A combination of at least 4 parameters seems to be highly optimal: an established tumor marker, a cytokeratin fragment, a marker of proliferation and a growth factor. The choice of an optimal combination of parameters differs depending on the histological type of tumor and the clinical diagnosis purpose (primary diagnosis, early detection of the disease, progression during the follow-up therapy monitoring, *etc.*).

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WHY SOMETIMES RESULTS OF TUMOR MARKERS STUDIES ARE SO VARIOUS? COMPARING OF RULES FROM CLINICAL RESEARCH STUDIES WITH STANDARDS OF NON-INTERVENTIONAL TUMOR MARKER STUDIES ADVANTAGES OF APPLICATION OF NEW STATISTICAL TOOLS

L. Pecen and O. Topolcan

[1]Academy of Science, Prague;
[2]Faculty Hospital and Medical School in Pilsen, E. Benese 13, 305 99 Plzen, Czech Republic

Why sometimes results of tumor markers studies are so various? Weaker rules and standards applied in non-interventional tumor marker studies are applied comparing them with rules from clinical research (new drug development). An application of strict rules could decrease the variability of published results. In clinical research it is required to define many important steps in advance (prior first patient enters into study) mainly the primary goal of the study. The primary goal includes a definition of the primary endpoint or endpoints and secondary endpoint(s). Only results concerning the primary endpoint will be analysed confirmatorily, secondary endpoint/s will be analysed exploratorily only. If more than one primary endpoints exist in the study, adjustment of statistical testing for multiple comparison has to be applied. It means alpha level for all confirmatory testing has to be 5% (0.05) in total for all comfirmatory testing. In clinical research the sample size has to be optimized with a specified significance level and power according selected primary endpoint/s and proposed study design. Variability of results will be decreased if null hypothesis and alternative hypothesis will be formally formulated in advance as well as statistical model will be selected in advance. Important issue is also the statistical monitoring of the quality of the data. Special emphasis has to be given to items entered manually into computer where recommended standard is double data entry by two independent persons and to compare results. Analysis of possible confounding and/or interacting factor could also help to explain difference between study' results and other

published papers with similar topic. Some promising new statistical tools (new in tumor markers analysis) as CART (Classification And Regression Trees) models, RECPAM (Recursive Partitioning and Amalgamation), Hidden Markov Chain Models, multiple events/endpoints Wei & Lachin-test *etc*.

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MICROIMAGING FT-IR OF HEAD AND NECK TUMOURS THE CASE OF SALIVARY GLAND PATHOLOGIES

C. Conti[1], P. Ferraris[1], E. Giorgini[1], C. Rubini[2], S. Sabbatini[1] and G. Tosi[1]

[1]Dipartimento di Scienze e Tecnologie Chimiche, [2]Dipartimento di Neuroscienze, Università Politecnica delle Marche, Ancona, Italy

The potential role of infrared (IR) spectroscopy in biomedical science has been exploited to distinguish different biomolecules by probing chemical bond vibrations and using these molecular and sub-molecular patterns to define and differentiate pathological from healthy samples. IR spectroscopy provides a spectral signature of the intensity and spatial location of the chemical components, so highlighting biochemical changes. FT-IR microimaging spectroscopy is particularly suitable to characterize tissue structures owing to the capability of generating maps or images of sample areas. The use of multivariate data and artificial neural network analysis can afford an unsupervised classification of biochemical components.

For a decade, we have been applying IR spectroscopy to study pathological states in breast, colon and, mainly, head and neck tissues characterizing various biochemical components with the aim to detect pathological states.

This contribution aims to further prove the potential of IR in isolating and defining characteristic spectral profiles in salivary glands attributable to various kinds of cancer: IR spectra of warthin tumour, oral epithelium with dysplasia, lymphoma, polymorphous low-grade adenocarcinoma, myoepithelioma, adenoid cystic carcinoma were compared with the corresponding healthy tissues. Using the intensity ratios 1660/1650 cm^{-1} of Amide I and II absorption bands, the $\nu_{asymCH_3}/\nu_{symCH_2}$ and $\nu_{asymCH_2}/\nu_{asymCH_3}$ bands and 1240/970 cm^{-1} as well as the absorption of the $C\text{-}O_{asym}$ stretching mode and the shape of the band at about 1080 cm^{-1} we found a realtionship with histological classification data.

In order to further verify the reliability of our classification, all the spectra from SK regions were mixed with those from zones characterized by other tumours obtaining a new HCA grouping in excellent agreement with their biochemical and histological characterization.

The results presented here show subtle biochemical and morphological changes distinguishing various kinds and grades of neoplasia in tissues from a specific region of the human body. The diagnosis of a pathology at a molecular level especially in the early stage is dependent on a defailed spectral analysis of infrared spectra, which contributes to the clinical histopathological and immuno-histochemical screening.

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EXOGENOUS ERYTHROPOIETIN MODIFIES TUMOR-INDUCED NEOANGIOGENESIS IN HUMAN TUMOR MODELS

József Tóvári

National Korányi Institute of TB and Pulmonology, Budapest, Hungary

Numerous clinical data suggest that hypoxia in tumors enhances the aggressiveness of a tumor and promotes malignant progression. Moreover, hypoxia is an independent prognostic factor in several types of malignant cancer. Correction of anemia and an increase oxygen level inside the tumor not only result in the improvement of quality of life but also enhance the success of cancer therapy, leading to improved survival of patients. Erythropoietin (EPO) has long been recognized as the major hematopoietic cytokine regulating normal erythropoiesis. However, recent studies indicated that, beside erythroid progenitor cells, tumor and endothelial cells express erythropoietin receptor (EPOR) as well, therefore recombinant human erythropoietin (rHuEPO) may affect their functions.

We reported that rHuEPO administration modulates tumor vasculature in human squamous cell and colorectal carcinoma xenograft models. *In vivo* rHuEPO treatment of xenografts at human-equivalent dose significantly increased the proliferation index of the tumor-associated endothelial cells and the size of CD31-positive intratumoral blood vessels. Moreover, rHuEPO administration resulted in reduced expression of vascular endothelial growth factor (VEGF) and hypoxia inducible factor 1-alfa (HIF-1α) but had no direct effect on the growth of EPOR-positive tumor xenografts. Due to the morphological alterations in tumoral microvessels, rHuEPO treatment led to a significantly improved efficacy of 5-fluorouracil (5-FU) chemotherapy. At the same time rHuEPO treatment significantly increased the efficacy of radiotherapy *in vivo*, mediated by increased tumoral blood vessel destruction.

Summarizing our data, rHuEPO treatment may modulate the efficacy of cancer chemo- and radiotherapy not only by reducing systemic hypoxia and tumoral HIF-1α expression,

but also by the regulation of intratumoral vessel formation and function.

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PIPOXOLAN INDUCES APOPTOSIS OF HUMAN U937 LEUKEMIA CELLS *VIA* MITOCHONDRIAL-MEDIATED PATHWAY

Huei-Yann Tsai[1,2,4], Jai-Sing Yang[2] and Yuh-Fung Chen[2,3,4]

[1]College of Pharmacy, [2]Department of Pharmacology and [3]Graduate Institute of Chinese Pharmaceutical Sciences, China Medical University, 91 Hseuh-Shih Road, Taichung, 40402, Taiwan;
[4]Department of Pharmacy, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 40402 Taiwan, ROC

We investigated the anti-leukemia effects of pipoxolan on the U937 leukemia cell line. Cell viability, reactive oxygen species (ROS) production, mitochondrial membrane potential, apoptosis induction, and caspases-1, -3 activity were examined by flow cytometry and caspase-activity assay. Apoptosis-associated Bcl-2 family proteins were examined by Western blotting. Our data showed that pipoxolan inhibited U937 cell proliferation in a dose- and time-dependent manner. The morphological assessment and cell cycle/apoptosis analysis by flow cytometry indicated that U937 leukemia cell line incubated with 10 μM pipoxolan induced cell apoptosis. Pipoxolan induced an increase of reactive oxygen species (ROS) in 1 h and thereafter a loss of mitochondrial membrane potential by flow cytometry. Pretreatment with *N*-Acetyl-L-Cysteine (ROS chelator) in pipoxolan-treated cells led to decline of ROS. We demonstrated an increase in the levels of Bax and decrease in the level of Bcl-2 and Bcl-xL, which were associated with the induction of apoptosis after 24 h treatment with pipoxolan in U937 leukemia cells. Our data suggest that pipoxolan could serve as a potent anticancer chemotherapeutic agent for human leukemia.

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PROTEOMICS IN CANCER RESEARCH

George Th. Tsangaris

Proteomics Research Unit, Center of Basic Research II, Biomedical Research Foundation of the Academy of Athens, Athens, Greece

Proteomics aim is to characterize, discriminate and identify the proteins in biological materials such as plasma and serum, urine, cell lines, tumors, biopsies etc in order to identify novel diagnostic biomarkers and therapeutic targets. The major proteomic technologies applied in cancer samples include two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and mass spectrometry (MS) while other methods including surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF)-MS, liquid chromatography combined to MS, isotope-coded affinity tag technology, reverse-phase protein arrays, and antibody microarrays are emerging as alternative proteomic technologies. Since there is little overlap between these approaches, the utility of these advanced technologies in cancer remains an elusive goal. Nowadays many proteomics studies have generated numerous datasets of potential diagnostic, prognostic, and therapeutic significance in human cancer. Despite the technological limitations of these technologies, there is little doubt that the proteomic approach has the potential to identify novel diagnostic biomarkers and therapeutic targets in cancer.

In accordance with the above statements the first part of that presentation refers to the proteomics technologies applied to the analysis of the entire proteome, to the identification and characterization of single protein molecules or protein complexes. The second part of the presentation refers to the most important areas that proteomics have contributed which are the prevention, diagnosis and treatment of cancer. The high throughput proteomic technologies applied lead to the elucidation of the pathways of carcinogenesis, to the characterization of specific biological markers for cancer diagnosis and to identification of new therapeutic targets. Furthermore, in order to answer clinical questions in cancer research, the accumulation of recent data is crucial to shift the emphasis of cancer proteomics from technology development to careful study design and data mining. The exploitation of these data will offer a deep knowledge of the mechanisms and the specific characteristics of the different types of the malignancy, greatly enhancing accurate prognosis and effective treatment.

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TOPOISOMERASE I PROTEIN EXPRESSION IN PRIMARY COLORECTAL CANCER AND RECURRENCES AFTER 5-FU-BASED ADJUVANT CHEMOTHERAPY

P. Gouveris, A. Lazaris, T. Papathomas, A. Nonni, V. Kyriakou, J. Delladetsima, ES. Patsouris, S. Tsaousi, J. Syrios, E. Patsouris and N. Tsavaris

Medical Oncology Unit, Department of Pathophysiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Objectives: Our aim was to investigate whether chemotherapy with 5-FU induces an alteration in the levels of topoisomerase I (topo I) in colorectal neoplastic tissues *Methods:* Twenty-five colorectal cancer patients were

included in the study; patients had undergone surgical resection of the primary tumor, received post-operatively 5-FU-based adjuvant chemotherapy and then suffered from recurrences. In a standard three-step immunohistochemical procedure, a monoclonal antibody to topo I was applied in both specimens from each patient (one from the primary location and a second one from the recurrence). Statistical analysis was subsequently performed. *Results:* Malignant cells from the recurrences displayed a statistically significant increase, concerning the levels of topoisomerase I, by comparison with the primary tumors (p=0.01). The increase in topo I levels did not demonstrate significant correlations with Duke's stage (Fisher's Exact Test p=0.496), differentiation grade (p=0.661), localization (p=0.072), patient sex (p=0.434), nor with relapse free interval (p=0.493). There was a statistically significant relationship between the age of patients and increase in topo I levels (p=0.011). *Conclusion:* Topo I expression may be part of the malignant cells' phenotype in recurrent colorectal carcinomas, suggesting a potential role for Topo I in the acquisition of a metastatic phenotype. The increase of topo I immunohistochemical status is likely to be attributed to 5-FU and given the fact that high levels of topo I correlate with sensitivity to camptothecin, advanced colorectal cancer patients seem to benefit from topo I targeted anticancer drug therapy.

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TOPOISOMERASE I AND IIα PROTEIN EXPRESSION IN PRIMARY COLORECTAL CANCER AND RECURRENCES FOLLOWING 5-FLUOROURACIL-BASED ADJUVANT CHEMOTHERAPY. IMPLICATIONS FOR NEW APPROACHES IN CHEMOTHERAPY FOR CANCER AND INFECTION

Nicolas Tsavaris[1], Elias Skopelitis[1], Andreas Lazaris[2] and Christos Kosmas[3]

[1]Medical Oncology Unit, Department of Pathophysiology, "Laikon" University General Hospital, Medical School, National and Kapodistrian University of Athens;
[2]First Department of Pathology, "Laikon" University General Hospital, Medical School, National and Kapodistrian University of Athens;
[3]Second Department of Medical Oncology, "Metaxa" Cancer Hospital, Piraeus, Greece

Human DNA topoisomerases I and II (topo-I and -II) are essential for vital cellular processes such as DNA replication, transcription, translation, recombination and repair. Following a chain of observations and pilot studies, we present the findings of a pioneer study, in which topo-I and -II expression was correlated with outcome after chemotherapy in primary and relapsed colorectal cancer. Patients with colorectal cancer that had recurred following surgery and adjuvant chemotherapy and WHO underwent a second operation were included in the present study. All had undergone surgical resection of the primary tumor and received post-operatively 5-FU-based (5FU+Leucovorin, Mayo Clinic regimen) adjuvant chemotherapy. Tumor tissue was collected at the initial operation from the primary tumor and at the time of recurrence (during the second operation following chemotherapy). All tissues samples were analyzed for levels of expression of both topo-I and topo-IIa using standard three-step immunohistochemistry on paraffin sections. Forty patients were included in the study. Levels of expression of topo-I and topo-II were higher in malignant cells from tumor recurrences compared to primary tumors (p=0.0001 for both). There was a statistically significant positive relationship between patients' age and levels of topo-I (p=0.011) and topo-II (p=0.011) expression. The study results reported here underscore the role of topoisomerase expression in colorectal cancer and suggest a potential role in tumor recurrence. This model could be further studied to include other forms of neoplasia and infection, in an effort to elucidate the development of chemotherapy drug resistance, thus optimizing treatment strategies and improving cancer patient care.

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HER2/NEU OVEREXPRESSING BREAST TUMORS WITH INCREASED PAKT HAVE POOR OUTCOME AND DEVELOP DRUG RESISTANCE

Jay Vadgama, Yanyuan Wu, Hezla Mohamed, Ram Chillar, Ishrat Ali, Sheila Clayton and Dennis Slamon

Divisions of Cancer Research and Training, Hematology/Oncology, Department of Medicine Charles R. Drew University of Medicine and Science, and David Geffen UCLA School of Medicine, Los Angeles, CA, USA

Introduction: Breast cancer patients with HER2/neu overexpression have poor outcomes, with a decrease in disease-free (DFS) and overall survival. The biology of HER2/neu overexpression in breast tumors in African-American and Latina women is poorly understood. The purpose of this study is to understand the clinical significance of activated Akt (phospho-Akt or pAkt) expression in breast tumors from African-American and Latina patients with corresponding tissue HER2/neu over expression. Cellular and molecular studies have shown that activation of the cell signaling phosphatidylinositol-3-kinase/Akt cascade *via* the HER2/neu and other receptor tyrosine kinases induces cell proliferation. *Materials and Methods:* A total of 234 African-American and Latina patients were selected retrospectively. From this group, 141 tumor tissue samples were analyzed for

tissue pAkt by immunohistochemistry (IHC). This cohort consisted of 46 HER2/neu-positive (3+ by IHC) and 95 HER2/neu-negative tumors. The prognostic value of activated tissue Akt in relation to HER2/neu over expression for DFS was determined. *Results:* Patients with low pAkt and HER2-negative tumors had the best DFS. As expected, patients with HER2/neu-overexpressing tumors with low pAkt had a decrease in DFS. Similarly, those with high pAkt and HER2-negative tumors also had poor DFS. However, those with an increase in both HER2 and pAkt had the worst DFS. An increase in pAkt was significantly associated with HER2/neu-positive and lymph node-positive breast tumors. Tumors with high HER2 and high pAkt were metastatic. Multivariate analysis demonstrated that, in addition to the common risk factors such as larger tumor size, lymph node involvement, estrogen receptor/progesterone receptor-negative tumors, and HER2/neu-positive tumors, overexpression of pAkt was significantly associated with a decrease in 5-year DFS. A decrease in DFS with an increase in pAkt was observed in both HER2/neu-positive and -negative groups. However, the DFS was similar in HER2/neu-positive/pAkt-negative and HER2/neu-negative/pAkt-positive groups. *Conclusion:* Our data suggest that there may be differences in tumor phenotypes within patients with HER2/neu-overexpressing breast cancer. The overexpression of pAkt may be a powerful prognostic marker for predicting DFS and overall survival of breast cancer patients.

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ANTITUMOR EVENTS IN ENDOCRINE TUMORS

Zs. Valkusz[2], M. Radács[1], A. Juhász[3], J. Jojárt[1], J. Gardi[2], J. Julesz[2], J. Molnár[4] and M. Gálfi[1]

University of Szeged, Faculty of Juhász Gyula Teacher Education, [1]Institute of Applied Natural Science, Department of Environmental Biology, Faculty of Medical School, [2]First Department of Medicine Endocrine Unit, [3]Department of Psychiatry, [4]Institute of Microbiology, Szeged, Hungary

Introduction: Persistent agents (*e.g.*: halogenated hydrocarbones) are cummulated in various biological organisms. These chemicals originate from industrial and agricultural section of the society. Chlorobenzenes have strenghtened stabilized structures responsible for dose dependent effects on human population. *Aim:* to investigate (1) the transformation activity of endocrine tissues (Adenohypophysis-/Adh/, Neurohypophysis – /Nh/) altered by different doses chlorobenzenes (2) the effects of *in vivo* and *in vitro* given antitumor agents (retinoic acid, krocin extract, interferon – β, algae -extract). *Methods:* Wistar rats (♂ 10 animals/group) were treated (by gastorintestinal tube) with 1 mg/bwkg → A chlorobenzenes mix (ClBM: /hexachlorobenzenes: trichlorobenzenes = 1:1/); 1 μg/bwkg ClBM → B; 0,1 μg/bwkg ClBM → C, for 30, 60 and 90 days. Our control system was absolute control – untreated – (AC); Positive control – treated by solvent /0,0015% ethanol/ of ClBM – (+C); Negative 1 control – treated by drinking water – (-C1), Negative 2 Control – stress control with gastric tube only – (-C2). In the treatment protocol the nutrition scheme was simulated a daily utilization of a gastric tube. At the end of treatment the morphology (number of tumors) and the function (hormones content –by RIA-) of Adh and Nh tissues were detected. The antiproliferative treatment *in vitro* included (10^{-6} M) retinoic acid (RA)/Sigma/, (1 μg/ml) crocus extract (CE), (1 μg/ml) algae extract (AE), (10^{-6} M) interferon-β (IF-β). Morphological analyses were carried out from paraffin cuts with Gills-Haematoxilin-Eosin stains. Protein was measured by the modified Lowry method. Hormones were detected by RIA methods. Statistical analysis of the data was made with the ANOVA program. *Results:* The ClBM A and B treatment tranformed the cells of Adh and Nh tissues. The RA>CE>AE>IF β showed antitumor effects on Adh and Nh. The hormone release was increased in the ClBM induced endocrine cell cultures. The antitumor agents could modify the increased hormone release. *Conclusion:* At the end of *in vivo* treatments no tumors were found in the pituitary tissues. However Adrenocorticotropin, Prolactin, Oxytocin and Vasopressin release were shown in the primary Adh, Nh cell cultures, in the cases of A_{30}, A_{60} and A_{90}. *In vivo* ClBM pretreatment caused cell transformation in A_{60}, A_{90}, B_{60} and B90. In these cases promotion and initiation effects of ClBM could be detected in the pituitary tissue.

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THE ANTIPROLIFERATIVE EFFECTS OF EXTRACELLULAR BIVALENT CATIONS ON PROLACTINOMAS

Zs. Valkusz[2], M. Radács[1], Gy. Nagyéri[1], J. Gardi[2], J. Julesz[2], J. Juhász[3], J. Molnár[4] and M. Gálfi[1]

University of Szeged, Faculty of Juhász Gyula Teacher Education, [1]Institute of Applied Natural Science, Department of Environmental Biology, Faculty of Medical School, [2]First Department of Medicine Endocrine Unit, [3]Department of Psychiatry, [4]Institute of Microbiology, Szeged, Hungary

Introduction: Endocrine cell transformation can be induced by disturbing the endocrine regulation (f.e.: chaotic feed-back), and can be initiated (f.e.: benz-c-acridine, chlorobenzenes) or/and promoted (f.e.: chlorobenzenes, electromagnetic field) with agents and energy. *Aim:* To investigate the behaviour of different origin Prolactinomas (P). *Methods:* Wistar rats were used. Untreated animals were the controls, the *in vivo* oestron-acetate (subcutaneous implantate 1 μg/bw/week; for 6 months) treated rats were the disregulated P. From the two groups the

adenohypophysis (Adh) and the prolactinomas (P) were separated by enzymatic and mechanical dissociations as primer Adh and P cell cultures. The Adh cultures were treated with (0.01 μ/ml) benz-c-acridine (BcAT), and (0.1 fg/mg protein) chlorobenzenes mix (hexachlorobenzene: trichlorobenzene=1:1) (ClBT) for 6 days. After the treatment the cell cultures showed tumorous formations (BcAT; ClBT). The cultures were examined for hormon secreting function (Adrenocorticotroph hormone: ACTH; prolactin: PRL; growth hormon: GH –by RIA-) and for cell biological markers (membrane fluidity /–with fluorescense anizotropy-/, Na^+-K^+-ATP-ase activity /-by spectrophotometric method-/, Mg^{++}-dependent ATP-ase activity /-with spectrophotometric method-/, cAMP content /-with Amersham KIT-/). The different cell cultures were treated with extracellular calcium and magnesium in lower than the physiologic concentration (0.25 mM).The data were analyzed by ANOVA system. *Results:* The hormonal secretion was increased in all treated animals (BcAT > ClBT > P) compared to controls. However, the use of low concentration bivalent cations (Ca^{2+}, Mg^{2+}) decreased the hormone-release P > ClBT > BcAT. The cell-markers were lower than in controls. *Conclusion:* The pituitary cells show different parameters depending on the type of transforming treatment. Antitumor effects of low concentration of bivalent cations can be observed in prolactinomas.

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CONTROL OF THE CELL CYCLE BY NITRIC OXIDE IN HUMAN NEUROBLASTOMA CELLS

Marlies Van de Wouwer and Antonio Villalobo

Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas, Arturo Duperier 4, E-28029 Madrid, Spain

Nitric oxide (NO) has been recognized as a bi-modal regulator of cell proliferation, inducing either an increase in the proliferative rate or the arrest of the cell cycle at low and high concentrations, respectively. Exposure of tumor cells to both endogenously produced and exogenously added NO has been shown to exert both anti-tumor and pro-tumor actions. Nevertheless, the potential therapeutic effects of NO in cancer are currently under intense scrutiny. To get further insight into the action of NO on cell proliferation, we tested the effect of an exogenous NO donor on the expression and activation/deactivation of different regulators controlling cell cycle progression in human neuroblastoma NB69 cells. We demonstrate in this work that the chronic exposure (17-19 h) of these tumor cells to 2,2'-(hydroxynitrosohydrazono)bis-ethanimine (DETA/NO), a slow NO releaser ($t_{1/2}$=20 h) belonging to the NONOate family, induces a decrease in the expression and/or the phosphorylation of the transcriptional repressor pRb, which controls the G1/S transition, and a decrease in the phosphorylation of two cyclin-dependent kinases, Cdk2 and Cdk1(cdc2). This is accompanied by a decreased expression of cyclins (Cdks regulatory subunits), particularly cyclins D1, A and B1. We have found a variable effect of DETA/NO on cyclin E expression, consistent with reports in other cells. In addition, NO-induced changes in the phosphorylation pattern of cyclin E were noticed, as well as a decrease in the phosphorylation of cyclins D1 and B1. DETA/NO treatment also induces a slight decrease in the expression of the Cdks inhibitors $p16^{Ink4a}$ and $p27^{Kip1}$, in contrast to the enhanced expression of $p21^{Cip1/Waf1}$. We have also observed NO-mediated activation of the tumor suppressor protein p53, which might account for the up-regulation of $p21^{Cip1/Waf1}$. Taken together, our results highlight some of the molecular mechanisms subjacent to the observed proliferative arrest of cells exposed to NO. The anti-proliferative role of NO does not appear to be limited to its well-known action controlling the G_1/S transition, affecting systems such as pRb, Cdk2 (with cyclin E as a partner), cyclin D (a Cdk4/Cdk6 partner), and $p21^{Cip1/Waf1}$, but also affects systems controlling the S phase and the G_2/M transition, such as Cdk2 (with cyclin A as a partner), Cdk1(cdc2), and cyclins A and B itself. Moreover, our results could point to the possible entry of cells in apoptosis because of the observed upregulation of p53. This could occur after arrest of the cell cycle whenever the exposure to NO is sufficiently high in concentration and prolonged in time.

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THE PRENYLATED CHALCONE XANTHOHUMOL INDUCES UNFOLDED PROTEIN RESPONSE, AUTOPHAGY, AND CELL DEATH IN BREAST CANCER CELLS BY PROTEASOME INHIBITION

Barbara Vanhoecke[1], Mireille Van Gele[2], Wim Martinet[3], Anne-Sophie Vercoutter-Edouart[4], Sofie Lust[5], Delphine Debruyne[1], Denis De Keukeleire[6], Fritz Offner[5] and Marc Bracke[1]

[1]Laboratory of Experimental Cancer Research, Department of Radiotherapy and Nuclear Medicine, [2]Department of Dermatology, [5]Department of Hematology, Ghent University Hospital, Ghent, 9000, Belgium, [6]Laboratory of Pharmacognosy and Phytochemistry, Ghent University, Ghent, 9000, Belgium;
[3]Division of Pharmacology, University of Antwerp, Wilrijk, 2610, Belgium; [4]Unity of Structural and Functional

Glycobiology, University of Lille 1, 59655 Villeneuve d'Ascq, France

In this study, we report a new proteasome inhibitor xanthohumol that induced endoplasmic reticulum stress, autophagy and apoptosis in a panel of human breast cancer cell lines but, remarkably, not in human pimary normal breast epithelial cells. Using a proteomic approach, we found that xanthohumol stimulated the transcription and expression of the ER chaperone GRP78 and the activation of the ER stress transducers PERK, IRE1 and ATF6. Sustained ER stress was associated with oxidative stress and apoptotic events such as processing of caspases, down-regulation of anti-apoptotic bcl-xL and mcl-1, and PARP cleavage. Susceptibility to xanthohumol-induced apoptosis correlated with GRP78 expression levels. Furthermore, treatment with xanthohumol triggered the formation of autophagosomes, as evidenced by monodansyl cadaverin staining, transmission electron microscopy and the conversion of LC3-I into the autophagosome-specific isoform LC3-II. We also found that xanthohumol was able to inhibit 20S proteasomal activity resulting in an accumulation of polyubiquinated proteins. Altogether, our results indicate that the endoplasmic reticulum and the proteasome are interesting targets for selective drugs against breast cancer.

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MICROARRAY ANALYSIS OF MCF-7 BREAST CANCER CELLS TREATED WITH 1,25-DIHYDROXYVITAMIN D_3 OR THE SECO-9,11-BISNOR-17-METHYL ANALOG WY1112

E. Vanoirbeek[1], G. Eelen[1], I. Beullens[1], M. Van Camp[1], K. Engelen[2], K. Marchal[2], P. De Clercq[3], R. Bouillon[1] and A. Verstuyf[1]

[1]Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Katholieke Universiteit Leuven, 3000 Leuven;
[2]CMPG-ESAT, Katholieke Universiteit Leuven, 3000 Leuven,
[3]Vakgroep Organische Chemie, Universiteit Gent, 9000 Gent, Belgium

The biologically active form of vitamin D is 1α,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$]. 1,25-$(OH)_2D_3$ functions through the vitamin D receptor (VDR). After dimerization of the VDR with the Retinoid X Receptor [RXR] receptor, the complex binds to vitamin D response elements [VDREs], recruits coregulators and the transcriptional preinitiation complex to initiate target gene transcription 1,25-$(OH)_2D_3$ is an important regulator of calcium metabolism (classical effects), but has also antiproliferative and prodifferentiating effects on both normal and tumorous cells (non-classical effects). Consequently, 1,25-$(OH)_2D_3$ is an interesting drug for the treatment of hyperproliferative disorders such as cancer. The therapeutic dose however causes severe side-effects such as hypercalcemia and hypercalciuria. For this reason, structural analogs of 1,25-$(OH)_2D_3$ such as the seco-9,11-bisnor-17-methyl analog, WY1112 have been developed with increased antiproliferative capacities .

To identify key regulatory genes involved in the antiproliferative action of 1,25-$(OH)_2D_3$ and to distinct the molecular activities of 1,25-$(OH)_2D_3$ and WY1112, total RNA was extracted from MCF-7 breast cancer cells that were treated with 1,25-$(OH)_2D_3$ or WY1112 and was used for microarray analysis. The experiments revealed that WY1112 induced the same set of genes as 1,25-$(OH)_2D_3$ but the induction level of the individual genes was higher. Microarray analysis did not reveal genes that were exclusively regulated by WY1112. Altered sensitivities to metabolism or changed VDR-coactivator interactions may account for its superagonistic characteristics.

Genes that were up-regulated can be divided into groups that are involved in proliferation and apoptosis (14%), gene transcription (13%), cytoskeleton organization (11%), immune respons (7%), transmembrane transport (6%), ubiquitination (4%), extracellular matrix (4%) and lipid metabolism (3%).

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THE EFFECT OF *UNCARIA* EXTRACT ON MOLECULAR BIOMARKERS IN PATIENTS WITH COLORECTAL CANCER

T. Varjas[1], F. Budán[1], Á. Ember[2], K. Amrein[1], Ő.P. Horváth[2], L. Illényi[2], D. B. Antonio[1], T. Dávid[4], P. Perjési[5], E. Pázsit[6] and I. Ember[1]

[1]Institute of Public Health, University Medical School of Pécs, Szigeti str. 12, H-7643 Pécs;
[2]Institute of Surgery, University Medical School of Pécs, Ifjuság str. 13, H-7624 Pécs;
[3]Institute of Otolaryngology Head and Neck Surgery, Faculty of Medicine, University of Pécs, Munkácsy str. 2, H-7621 Pécs;
[4]LtdCoD™ Kft., Vörösmarty str. 15, H- 9155 Lébény;
[5]Institute of Pharmaceutical Chemistry, University of Pécs School of Medicine, Rókus str. 2, H-7623 Pécs, Hungary;
[6]Hospital And Clinic of Diósgyőr, Obstetrical Ward, H-3520, Miskolc, Hungary

We investigated the combined effect of surgical treatment and the influence of consumption of CoD™ tea (which contains *Uncaria guianensis* and *U. tomentosa* also known as cat's claw bark extract) on the expression of c-*myc, Ha-ras, Bcl-2, Ki-ras* and *p53* key onco/suppressor genes and CA199, CEA tumor markers in blood samples of patients with colorectal cancer (CRC) in a "one-year follow up study". These key onco/suppressor genes are good molecular epidemiological

biomarkers of the exposure carcinogenic agents or early biological effects in peripheral blood and are also good biomarkers of potential chemopreventive effect as well. Their expression enables the effect of surgical treatment combined with neoadjuvant chemotherapy to be followed and may predict the outcome of carcinoma. Moreover, their expression can show the possible additional curative/preventive effect of CoD™ consumption. The scavenger type antioxidant capacity of blood was assessed by deoxyribose degradation test. Blood samples were taken at the day of surgical treatment, then 1 week, 3 and 6 months and 1 year later, by which time patients were consuming 0.2 l standard portion of CoD tea three times a day. There were 2 groups of 20 individuals: CRC patients (CoD™ consumers) and a control group of healthy individuals (they were not consumers of CoD™). Before surgical treatment all CRC patients received Ca-folate and 5-FU and 45 Gy radiation therapy; after the resection they were treated according to de Gramont protocol. Surgical treatment and neoadjuvant therapy are able to suppress the expression of *c-myc, Ha-ras, Bcl-2, Ki-ras, p53* onco/supressor gene FOR 12 months. Moreover CoD™ tea together with conventional treatment caused a strong and significant decrease of the expression of c- myc and Ha- ras oncogen in comparison with the nonconsumer controls.

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THE MYELODYSPLASTIC SYNDROME – QUESTIONS AND ANSWERS. THE BUDAPEST STUDY GROUP EXPERIENCE

J. Varkonyi, P. Farkas, G. Varga, G. Kollai, G. Szombath, A. Tordai, H. Andrikovics, B. Schoket, I. Karádi, K. Kádár, G. Füst and Z. Prohászka

Semmelweis University, Budapest, Hungary

The myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid stem cell disorders characterized by refractory cytopenias, previously known as preleukemia. In the primary form, the etiology is still unknown. However, the multiple hit pathogenesis is considered valid for this condition, and provides a possible explanation for the evolution of refractory cytopenia into acute leukemia.

In an attempt to find answers to questions of MDS pathogenesis, the Budapest Study Group has compared certain genetic features of patients with multiple myeloma (MM), a B-cell malignancy with a similar prevalence in the elderly with those of MDS.

We analyzed approximately 100 patient samples from both conditions for genetic polymorphism regarding the metabolizing enzymes GST especially those participating in the detoxification of carcinogens. We also compared the incidence of mutations of the hemochromatosis gene (*HFE*, C282Y and *H63D*), and *IL-6*/*IL-6R* and *TNF-α* polymorphisms.

We found a significant difference in incidence of only the *HFE* gene polymorphism between the two groups, with a higher mutation rate in the MDS group, whereas the *HFE* mutation was even less frequent in the MM group compared to healthy controls.

That the *HFE* gene mutation has an impact on iron absorption, even in its heterozygote form, is a well known phenomenon. Iron excess confers oxidative stress tissues leading to bone marrow impairment. On the other hand, prolonged uncorrected deficiency states, such as low copper levels and vitamin B12 deficiency, might be as harmful for tissues with a high cell turnover such as bone marrow stem cells. Iron overload or other toxic compounds injuring mitochondria, or deficiency in trace elements and vitamins with importance in cell respiration, might both give rise to more primitive cells – thus conferring a selection advantage for them. The literature includes data to support this concept.

According to this model, one should question the priority of the genetic events and consider them as secondary to an unbalanced metabolism that instead can be considered as the primary etiological factor in the disease pathogenesis in a certain group of MDS patients.

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SUN EXPOSURE AND USE OF SOLARIUMS DURING THE FIRST FIVE DECADES OF LIFE AND RISK OF CUTANEOUS MALIGNANT MELANOMA: THE NORWEGIAN-SWEDISH WOMEN'S LIFESTYLE AND HEALTH COHORT STUDY

Marit B. Veierød[1], Hans-Olov Adami[2,3], Eiliv Lund[4], Bruce Armstrong[5] and Elisabete Weiderpass[2,4,6,7]

[1]Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway;
[2]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden;
[3]Department of Epidemiology, Harvard School of Public Health, Boston, MA;
[4]Institute of Community Medicine, University of Tromsø, Tromsø, Norway;
[5]School of Public Health, University of Sydney, Sydney, Australia;
[6]Department of Etiological Research, Cancer Registry of Norway, Oslo, Norway;
[7]Department of Genetic Epidemiology, Folkhälsan Research Center, Helsinki, Finland

Background: Sun exposure is the major established and modifiable risk factor for cutaneous malignant melanoma. Use of indoor tanning equipment has also been associated with increased risk, but less consistently. The importance of exposure to ultraviolet (UV) radiation in different periods of life and possible effect modification by an individual's sensitivity to UV exposure is not completely understood.

Materials and Methods: The Norwegian-Swedish Women's Lifestyle and Health Cohort Study included more than 100 000 women aged 30-50 years in 1991/92. Information on sun exposure, use of a solarium (*i.e.* a sunbed or sunlamp) and individual characteristics were collected at cohort enrolment through a self-administered questionnaire. Frequency of sunburns, sunbathing vacations and use of a solarium were recorded for the age periods 0-9, 10-19, 20-29, 30-39 and 40-49 years. Complete follow-up is achieved by linkage of the study database to national registries in Norway and Sweden. Relative risks are estimated by Poisson regression. *Results:* The first results from this cohort study were published in 2003 (Veierød *et al*. J Natl Cancer Inst *95*: 1530-1538, 2003) and included follow-up through 1999 *i.e*. a short follow-up period to estimate the effects of midlife exposure. We present now new results with follow-up through 2005. A total of 106,366 women were followed up to14 years and 412 incident cases of cutaneous malignant melanoma were reported to the national Cancer Registries. The mean age at diagnosis was 49 years. A twofold increased risk of melanoma was found for sunburns in childhood, teenage years and early adulthood (≥2 sunburns per year *vs*. none). In this cohort, solarium use in childhood and teenage years was rare. Solarium use in early adulthood and midlife increased the risk of melanoma. We will also discuss the potential impact of national regulations on indoor tanning devices implemented in 1982/83 resulting in changes in the UVB to UVA ratio.

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TARGETING CANCER CELLS BY AN OXIDANT-BASED THERAPY

J. Verrax, R. Beck, C. Lannoy, H.S. Taper and P. Buc Calderon

PMNT unit, School of Pharmacy, Université Catholique de Louvain, 73 Avenue E.Mounier 1200 Brussels, Belgium

Among all the different features of cancer cells, two are of particular interest: their nearly universal glycolytic phenotype and their sensitivity towards oxidative stress. By using the combination of menadione (vitamin K3) and ascorbate (vitamin C), we took advantage of these features to develop an original approach that consists of the exposure of cancer cells to an oxidant insult. The results we obtained can be summarized as follows:

The combination exhibits a synergistic antitumor activity *in vivo* as well as *in vitro* through the potentiation of the menadione redox cycling by ascorbate, resulting in the generation of reactive oxygen species (1, 2). Importantly, non-transformed cells are not affected by this treatment, likely because of their better antioxidant defences.

The oxidant insult rapidly induces DNA strand breaks which provoke the activation of an enzyme participating in DNA repair, poly(ADP-ribose) polymerase (PARP). Activated PARP consumes cytosolic NAD^+, and because NAD^+ is required for glycolysis, this render cells unable to use glucose as a metabolic substrate. Due to the high energetic dependence of cancer cells on glycolysis, this leads to an energetic crisis which ultimately results in necrotic cell death (3).

Interestingly, the oxidative stress provoked by the ascorbate/menadione combination also induces the disruption of the chaperone function of Hsp90, resulting in the degradation of important oncogenic proteins essential for cancer cell survival, such as Bcr-abl. This phenomenon is only observed in tumour cells and represents a second pathway that participates in the anticancer activity of the combination.

Taken together, our data suggest that the combination of ascorbate and menadione could represent a new and interesting auxiliary cancer therapy. Since both compounds are of low toxicity, they can easily be included in a classic anticancer protocol without any supplementary risk for the patients, as shown in the recent phase I/IIa study performed in prostate cancer patients who failed standard therapy (4).

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CARDIAC HORMONES: NEW ANTICANCER AGENTS

David L. Vesely

University of South Florida Health Sciences Center/VA Hospital, Tampa, Florida, USA

Cardiac hormones are a family of peptide hormones synthesized mainly in the atrium of the heart. One gene in the heart of this peptide hormone family synthesizes a 126 amino

acid (a.a.) prohormone which contains four cardiac peptide hormones consisting of a.a. 1-30 (long-acting natriuretic peptide, LANP), a.a. 31-67 (vessel dilator, VDL), a.a. 79-98 (kaliuretic peptide, KP) and a.a. 99-126 (atrial natriuretic peptide, ANP).

These peptide hormones decrease in up to 97% of human pancreatic, breast, colon, ovarian, kidney and prostate adenocarcinomas, glioblastomas of the brain and melanomas, as well as small-cell and squamous cell lung carcinoma cells *in vitro*.

When infused subcutaneously for 28 days with fresh hormones weekly at 3 nM min^{-1} kg^{-1} body weight in athymic mice bearing human pancreatic adenocarcinomas, they eliminate up to 80% of the human pancreatic adenocarcinomas. Even in the treated animals which did not have a total elimination of the pancreatic carcinoma, their tumor volume decreased to less than 10% (and with vessel dilator to 2%) of that of the untreated animals and the treated animals achieved a normal lifespan.

Similarly, these peptide hormones eliminate two-thirds of human breast adenocarcinomas growing in athymic mice without any surgery. The mechanism(s) of action cardiac of these hormones in cancer cells includes a 97% inhibition of the phosphorylation of extracellular-signal regulated kinases 1/2 (ERK 1/2) and of the upstream mitogen-activated protein kinases (MEK 1/2). These inhibitions are mediated by the intracellular mediator cyclic GMP. The final step in their mechanism of action is a strong ability to inhibit DNA synthesis within the nucleus of the cancer cells, also mediated by cyclic GMP.

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MYZITIRAS AND VTHELA MORPHOTYPES AS NEW SIGNATURES OF NEOPLASTIC PROGRESSION?

Ondřej Tolde[1], Daniel Rösel[1], Jan Brábek[1] and Pavel Veselý[2,3]

[1]Department of Cell Biology, Faculty of Science, Charles University in Prague, Prague;
[2]Institute of Molecular Genetics AS CR Prague 6, 166 37;
[3]Prague Burn Centre, 3rd Medical Faculty, Charles University, Prague 10, 100 00 Czech Republic

EM-G3, a new clonal cell line (1) was derived from a primary lesion of human infiltrating ductal breast carcinoma and shown to originate with high probability from common progenitors of luminal and myoepithelial cells that were immortalized in an early stage of tumorigenesis. Such presumably low malignant cells attract efforts focused on *in vitro* induction of neoplastic progression. If this is successfully achieved new traits/markers of malignancy could be revealed. With this aim in mind EM-G3 have been *in vitro* exposed to chronic nutritional stress that was at the beginning intermittent and supported by two subsequent treatments of an activator of the protein kinase C, 12-O-tetradecanoylphorbol-13-acetate (TPA). After six month of this challenging procedure resulting G3S1 cells grew in standard culture medium without growth factors needed for EM-G3 cells. Both cell populations were then compared. It was found that in Matrigel invasion chambers G3S1 cells exhibited ca 2.5 fold enhancement of the invasion capacity over a 24 h period as compared to parental EM-G3 cells. Also the degradation of FITC-gelatine was substantially increased in G3S1 cells. F-actin rich structures resembling podosomes were present in both EM-G3 and G3S1 cells and to considerable extent colocalized with the sites of degradation. These podosome-like structures were enriched in phosphotyrosine; however, they did not contain phosphorylated cortactin suggesting they are at least in part different from podosomes. Interestingly, in some small holes in gelatine indicating their early stage, the F-actin structures were observed hinting that there could be only a slight difference between invadopodia and podosomes consisting in an availability of a penetrable substrate. Examination of G3S1 cells nascent morphology one hour after seeding in HBSS on gelatine covered coverslip revealed an increased incidence of myzitiras (sucker) and vthela (leech) morphotypes described earlier (2). Coincidence of apparent neoplastic progression of G3S1 cells with the appearance of these distinguished morphotypes leads to a possibility of exploiting these morphological traits in selection of individual cells for targeted examination of gene expression. Such an approach can enrich our knowledge of mechanisms of neoplastic progression.

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LOW-LEVEL RADIATION AND CANCER

A.C. Vikis

3251 Carriage Hill Place, Ottawa,
Ontario, Canada K1T 3X6

The medical applications of radiation are the largest source of radiation exposures, following natural background radiation. They include diagnostic radiology, radiotherapy, nuclear

medicine and interventional radiology. Ionizing radiation (x-rays, alpha, beta, gamma) is known to cause chemical changes to biological molecules, which may lead to cancer. Damage to DNA, particularly double-strand breaks is the main initiating event; however, damage to other cellular components may influence cell functioning and progress to the malignant state. Sources of information about the health effects of ionizing radiation include theoretical and experimental studies (including animal studies) of the underlying science, especially biology, and epidemiological studies of humans exposed to radiation. This presentation will examine the current understanding of the harmful effects of ionizing radiation. Special emphasis will be given to low levels of ionizing radiation, below about 100 mSv, where there is considerable uncertainty of the risks and, in some cases, there have been claims of likely beneficial effects.

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GENETIC SUSCEPTIBILITY TO LUNG CANCER

Haris G. Vikis[1], Pengyuan Liu[1], Ming You[1], Marshall W. Anderson[2] and Genetic Epidemiology of Lung Cancer Consortium

[1]Washington University School of Medicine, St. Louis, Missouri; [2]University of Cincinnati, Cincinnati, OH, USA

Three recent genome-wide association studies identified associations between markers in the chromosomal region 15q24-25.1 and the risk of lung cancer. We conducted a genome-wide association analysis to investigate associations between single-nucleotide polymorphisms (SNPs) and the risk of lung cancer, in which we used blood DNA from 194 case patients with familial lung cancer and 219 cancer-free control individuals. We identified associations between variants on chromosomes 1, 3, 6, 9, 12, 15, and 20. Here we describe common sequence variants on 15q24-25.1 (which spanned LOC123688 (a hypothetical gene), PSMA4, CHRNA3, CHRNA5, and CHRNB4) and lung cancer. The risk of lung cancer was more than five-fold higher among those subjects who had both a family history of lung cancer and two copies of high-risk alleles rs8034191 (OR=7.20, 95% CI=2.21 to 23.37) or rs1051730 (OR=5.67, CI=2.21 to 14.60) that were located in the 15q24-25.1 locus, than among controls. Thus, further research in elucidating causal variants in the 15q24-25.1 locus that are associated with lung cancer is warranted.

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CANCER DIAGNOSIS BASED IN THEDIFFERENTIAL EXPRESSION OF A NOVEL FAMILY OF NON CODING MITOCONDRIAL RNAS

J. Villegas[1,2,3], V. Burzio[1,2,3], E. Landerer[1,3], E. Villota[2,3], C. López[1], S. Vidaurre[1], M. Araya[1], A. Rivas[1], L. Cruz[1] and L.O. Burzio[1,2,3]

[1]Andes BioScience S.A., Fundación Ciencia para la Vida, Fundación Instituto Milenio (MIFAB), [2]Grupo BIOS, [3]Facultad de Ciencias de la Salud, Universidad Andrés Bello. Santiago, Chile

We describe a novel transcript which is overexpressed in human proliferating cells but not in resting cells. The transcript contains a hairpin structure comprising an IR of 815 nt linked to the 5' end of the 16S mtrRNA, forming a long double-stranded structure. This novel transcript is a non-coding RNA (ncRNA) and experimental evidence suggests that the transcript is synthesized in mitochondria. Therefore, we named this transcript "sense non-coding mitochondrial RNA" (sncmtRNA). The expression of this transcript can be induced in resting lymphocytes by stimulation with phytohaemagglutinin (PHA). On the other hand, proliferating cells treated with aphidicolin repress the expression of the transcript. The cells resume proliferation if the drug is removed and over-express the ncmtRNA again. These results suggest that this ncmtRNA may play a role in cell proliferation. *In situ* hybridization (ISH) using oligonucleotide (ODN) probes revealed that the transcript is expressed in tumor cells from biopsies and also in tumor cell lines from a variety of human cancers, but is not present in normal quiescent cells. However, to our surprise, normal proliferating cells also express another transcript, named antisense ncmtRNA (AsncmtRN). The differential expression of the sense and antisense ncmtRNAs allow for the selective discrimination between normal proliferating, quiescent and tumor cells. The study performed in a hundred of biopsies suggests that these molecules could constitute a new and specific biomarker.

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MICROFLUIDIC MAGNETIC CELL SORTING SYSTEM FOR CTC DETECTION AND TYPING

A.-E. Saliba[1], L. Saias[1], J. Salamero[1], V. Fraisier[1], J.-Y. Pierga[2], F.C. Bidard[2], C. Mathiot[2], P. Vielh[3], F. Farace[3] and J.-L. Viovy[1]

[1]Institut Curie, Section de Recherche, [2]Institut Curie, Section Médicale, and [3]Institut Gustave Roussy, UPRES EA3535, France

We present a new integrated microfluidic system for the high throughput, high content sorting and analysis of cancer cells.

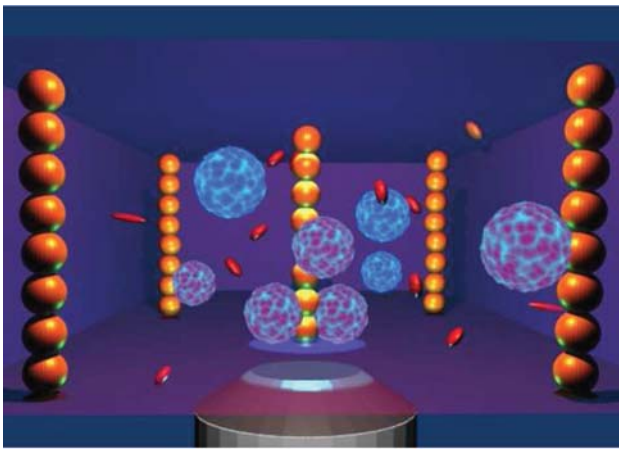

Figure. *Principle of the method: a microfluidic channel with microfabricated magnetic domains, on which columns of magnetic beads bearing antibodies directed toward cancer cells surface antigens (orange) self-assemble under a magnetic field. The sample containing cancer cells (reddish blue) and normal haematopoietic cells (light blue) is then flown in the channel. Upon collision with the magnetic columns, the cancer cells are captured and normal cells resume their motion.*

It is able to capture tumour cells with specific antibodies, like circulating tumour cells in whole blood or cells from Fine Needle Aspirates, with high specificity and efficiency. Cell separation in this system, is based on a new concept developed in our group (1). It involves an immunoaffinity separation matrix made of magnetic beads, self-assembled onto a pattern of magnetic traps prepared by microcontact printing (2) (Figure).

Recent work (3) did show that the microfluidic format is able to considerably enhance the yield of CTC capture, opening new routes for diagnosis, prognosis of metastatic relapses, and selection of the most adapted treatment based on a molecular typing of the CTC. In addition to the high efficiency described in this earlier work, in our new system the captured cells can be visualized using the most sophisticated tools of cell microscopy, allowing for extensive automated phenotyping by multicolour antibody labelling and morphological analysis. In a near future, the technology will also allow genotyping. Quantitative characterization of the efficiency and specificity of capture, obtained with model cell lines, and our first applications to cancer cell typing from patient's blood samples will be presented in the conference.

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STATINS AND CANCER – ARE ALL STATINS THE SAME?

Libor Vítek

Institute of Clinical Biochemistry and Laboratory Diagnostics and 4th Department of Internal Medicine, 1st Faculty of Medicine, Charles University in Prague, Czech Republic

Despite supporting theory as well as experimental evidence, there is still clinical controversy as to whether statins can prevent from cancer. These contradictory views are based on large epidemiological studies and their meta-analyses which, however, have been focused primarily on cardiovascular outcomes. In addition to these limitations, there are also other reasons to account for different anticancer effects of statins. These are related to the physico-chemical properties of statins (lipophility/hydrophility), as well as to different pharmacokinetic and pharmacodynamic properties. In fact, we were able to detect large differences in antiproliferative effects on experimental pancreatic cancer among statins used for clinical purposes. The least efficient statins in our studies were pravastatin and atorvastatin, while rosuvastatin, cerivastatin and fluvastatin were the most potent compounds.

These data may account for inconclusiveness of cancer prevalence/incidence among statin users in cardiovascular trials. Provided that pravastatin might be the least efficient statin, recent meta-analytic studies might have been confounded by pravastatin trials. This, indeed, is the case for meta-analysis of 7 trials by Hebert *et al*. [JAMA 1997], where 3 out of 7 studies involved were pravastatin trials. The same is true for a study by Bjerre *et al*. [Am J Med 2001] (3 out of 5 studies involved), CTT Collaborator's study [Lancet 2005] (5 out of 14 studies involved), as well as meta-analysis by Dale *et al*. [JAMA 2006] (10 out of 20 studies).

It is clear that further large and well-designed epidemiological studies are definitely needed to determine any potential chemopreventive role of individual statins.

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POSSIBLE INFLUENCE OF *NQO1* POLYMORPHISMS IN THE DEVELOPMENT OF HPV-RELATED CERVICAL CANCER

Milene Volpato

Section of Experimental Therapeutics, Leeds Institute for Molecular Medicine, Wellcome Trust Brenner Building, St-James University Hospital, University of Leeds, Leeds LS9 7TF, UK

NAD(P)H:Quinone oxidoreductase 1 (NQO1) has multiple functions in cells. It is best known for its involvment in

detoxification processes. However, it was more recently shown to play a role in p53 *via* an mdm2-independent route. Such interaction of NQO1 with p53 can partially abrogate p53 degradation by human papilloma virus (HPV) protein E6. There are two characterised polymorphic variants of *NQO1*, namely NQO1*2 and *3, which cause loss of NQO1 activity, raising the possibility that individuals harboring these variants may have reduced p53 activity and increased susceptibility to HPV-mediated cervical cancers. A study was therefore designed to survey *NQO1* polymorphism incidence in a population of patients with pre-neoplastic and cancerous lesions of the cervix.

Genomic DNA was extracted from histological blocks of cervical cancer (n=90) and precancerous CIN lesions (n=293) and *NQO1* polymorphic status determined by real-time PCR. No significant difference existed between the distribution of NQO1*2 and p53*72 in either patient group or the distribution reported in healthy individuals. In contrast, a significant increase in the incidence of the mutated form of the NQO1*3 polymorphism with a risk ratio of 2.53 (95% conf. interval=3.52, $p<0.0001$) was observed in patients with cervical cancer. Additionally, no relationship was established between the *NQO1* polymorphic variants and the CIN grade of the patients.

The higher incidence of the NQO1*3 polymorphism observed within the cancerous group compared to both healthy and pre-cancerous groups was surprising as variations in the NQO1*2 incidence have been previously reported in various cancer types but no association had been established between NQO1*3 polymorphism and cancer risk to date. Whilst the mechanistic basis for this observation is not known, this study suggests that individuals who harbour NQO1*3 may be at greater risk of progressing from preneoplastic to malignant cervical cancer. If confirmed in further experiments, such result scould provide a basis for implementing *NQO1* polymorphism screening of patients with dysplasia of the cervix.

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PRESERVATION OF GAMETES IN GYNECOLOGICAL CANCERS

Sören von Otte and Safaa Al-Hasani

Department of Obstetrics and Gynecology, University of Schleswig-Holstein, Campus Luebeck, Ratzeburger Allee 160, 23538 Luebeck, Germany

With an increase in the efficacy of anticancer therapy and quite efficient early diagnosis in gynecological cancer, increased long-term survival of cancer patients and long-term complications of anticancer treatments are being encountered (1). The lost of ovarian reserve or function due to gonadal toxicity is a major problem.

A wide range of new fertility preservation options and/or techniques, although the majority of them are experimental, are now available for female gamete preservation prior to oncological treatments (2). Ovarian transposition, a surgical preservation technique, is one of these methods that prevents gonadal toxicity due to radiotherapy. Partial and/or total (experimental) extraction, subsequent cryopreservation and re-transplantation of ovarian tissue, another surgical preventive option both for radiotherapy and chemotherapy side-effects, is expected to be a paramount method in the future that was performed in selective cases previously (2-4). In particular, medical methods with such agents as GnRH analog or antagonists, and Danazole have been reported to prevent chemo or radiotherapy related gonadal toxicity. Cryopreservation of oocytes, zygotes and embryos either by vitrification or slow-rate freezing subsequent to ovarian stimulation and oocyte pick-up, along with/without traditional IVF and intracytoplasmic sperm injection are other common preventive techniques (3, 4).

Currently cryopreservations of the oocyte, zygote and/or embryos seem to be the most effective methods in fertility preservation, especially in women of reproductive age (5). In regard of the cryopreservation techniques vitrification has been claimed as the most appropriate and efficient technique due to simplicity and cost-effectiveness (6, 7). On the contrary, surgical methods are still accepted as difficult, and costly alternatives, as well experimental ones such as re-transplantation are not reported to be significantly efficient and reliable alternatives. In parallel, the effects of medical preventive alternatives, those generally resulting in a pseudo-menopausal status and mainly recommended to patients prior to child-bearing, are still controversial concerning side-effects, reduced effectiveness.

The number of available techniques for conserving fertility has increased in the last decade, but large studies are still needed to draw any conclusions. Some of these effective options such as vitrification of embryos is not allowed in Germany. Other technical issues remain unresolved, as is the question of medical insurance reimbursement for the most efficient procedures. All current aspects and techniques should be compared in order to define the best method and standardization in female fertility preservation. New approaches and current management on fertility preservation in gynecological cancer were discussed in this presentation.

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MULTIPLE MARKERS IN THE FOLLOW-UP OF OVARIAN CANCER

Jindra Vrzalová, Ondřej Topolčan, Markéta Pražáková, Miroslava Časová and Zdeněk Novotný

Teaching Hospital and Medical Faculty in Pilsen, E. Benese 13, 305 99 Plzen, Czech Republic

Introduction: Gil Mor *et al*. developed after a proteomic study a multiplex panel for ovarian cancer including Macrophage Migration Inhibitory Factor (MIF), prolactin, CA-125, leptin, osteopontin (OPN) and a IGF-II. *Aim:* To use this multiplex panel for follow up monitoring of ovarian cancer patients. *Methods:* Two groups of patients were studied: Control gr. 0: pat. with benign ovarian cyst (20 patients) and patients with ovarian carcinoma, stage III-IV (19). Serum levels of biological activity markers of ovarian carcinoma were measured by Beadlyte® Human Cancer Biomarker Panel kit from Millipore-Upstate (USA) and Luminex 100 instrument (Luminex corp., USA). Simultaneously, levels of other markers (CA125, TK, TPS, HE4 and Monototal) were measured by routine immunoanalytic methods. *Results:* From the multiplex markers, the best ROC characteristics were observed for CA125, IGFII and OPN. HE4 marker had the best ROC characteristic from all measured markers. In the multiplex panel, significant differences (Wilcoxon test) in marker levels between groups were found only for CA125 (higher level in carcinoma), OPN (higher level in carcinoma) and IGFII (lower level in carcinoma). *Conclusion:* Multiplex analysis enables an easy simultaneous measurement of multiple markers and so enables the use of a scoring system in future. Supported by the research project VZ MSM 0021620819.
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WHEN HISTOLOGY MEETS SYSTEMS BIOLOGY

Mathias Wagner[1], Frank Weichert[2], Andreas Groh[3], Sally Said Kamel Awad[4], Ali Shamaa[5], Tereza Richards[6], Roland Linder[7] and Constantin A. Landes[8]

[1]Institut fuer Pathologie, Universitaet des Saarlandes;
[2]Informatik VII, Technische Universitaet Dortmund;
[3]Institut für Angewandte Mathematik, Universitaet des Saarlandes, Germany;
[4]Department of Maxillofacial Surgery, University of Mansoura;
[5]Department of Oral Biology, Minia University, Egypt;
[6]University of the West Indies, Kingston, Jamaica;
[7]Institut für Medizinische Informatik, Universität zu Lübeck;
[8]Klinik fuer Mund-, Kiefer- und Plastische Gesichtschirurgie, Johann Wolfgang Goethe-Universitaet Frankfurt am Main, Germany

Computer generated cells and tissues may be used to predict biological behaviour for example of cancer. Virtual objects can be created by the employment of a variety of approaches such as Quaoaring. A systematic evaluation of these steps leading to applicability in systems biological mathematical modeling is overdue. A preliminary overview and comparison of methods however can be given. The results of such a survey suggest that to date no single approach meets all requirements in systems biology. Visions of developing a gold standard therefore remain far off which might have considerable impact on the possible use of computer generated cells and tissues in the context of quality control in systems biological mathematical modeling. Ongoing and future studies should conclusively address this issue.

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TARGETING OF TUMORS WITH RADIOLABELED VITAMINS

Robert Waibel, Cristina Müller, Thomas L. Mindt, P. August Schubiger and Roger Schibli

CRS, Paul Scherrer Institute, CH-5232 Villigen PSI, Switzerland

Rapidly growing cells show an increased demand for nutrients and vitamins. We exploited the supply route of vitamin B12 and folate to deliver radiolabeled derivatives to hyperproliferative cells. However, the tumor uptake of vitamin B12 and folate-based radiotracers was shown to be low compared with the high renal retention of radioactivity. For clinical application of radiopharmaceuticals, it is known

that high kidney uptake of radiolabeled compounds may lead to radiation toxicity.

In order to obtain better tumor to non tumor ratios, we interfered with the biological properties of these vitamins to be stored in normal organs like liver and kidney.

For vitamin B12 targeting, we designed new vitamin B12 conjugates that do not bind to their main transport protein transcobalamin II (TCII) and thus tumor to background ratios were tremendously increased.

For radiofolate targeting preadministration of the antifolate drug Pemetrexed led to an almost complete blockade of radioactivity uptake in folate receptor positive kidneys whereas the tumor uptake remained unchanged and thus tumor to kidney ratios were significantly improved. Obviously, Pemetrexed interacts differently with the uptake mechanism of the ^{99m}Tc-folate on the tumor tissue and kidneys respectively.

In vivo SPECT/CT studies in mice demonstrated the specific accumulation of ^{99m}Tc-PAMA-vitamin B12 and 99mTc-PAMA-folate in tumors and an almost complete absence or significant reduction of radioactivity in the renal tissue. Clinical trials to prove these concepts started this fall in collaboration with the University Hospital Zurich.

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INTAKE OF RED WINE POLYPHENOLS PREVENTS TUMOR GROWTH AND NEOVASCULARIZATION IN A SYNGENIC MODEL OF COLON CANCER

Allison Walter[1], Nelly Etienne-Selloum[1], David Brasse[2], Hadeel Khallouf[1], Alain Beretz[1] and Valérie B. Schini-Kerth[1]

[1]Institut Gilbert-Laustriat, UMR 7175 CNRS, Université Louis Pasteur, Département de Pharmacologie et Physicochimie, Faculté de Pharmacie, 74 route du Rhin, 67401 Illkirch;
[2]Pluridisciplinaire Hubert Curien, UMR 7178 CNRS-IN2P3, Université Louis Pasteur, 23 rue du Loess, 67000 Strasbourg, France

Introduction: The growth of a tumor and its ability to develop metastases is angiogenesis-dependent. In Europe, colon cancer is the second and the third cause of mortality due to cancer in women and men, respectively, and it was estimated that an effective nutritional prevention, namely to eat more fruit and vegetables, would prevent more than 60 to 80% of colon cancer. Since epidemiological studies have indicated that a moderate and regular consumption of wine is associated with a reduced risk of cancer, we hypothesize that red wine polyphenols (RWPs) may prevent tumor growth by controlling tumor angiogenesis. *Methods:* C26 cells, derived from colon carcinomas (chemically induced in BALB/c mice), were subcutaneously injected in each flank of 9-week-old BALB/c mice. Two days after the injection, RWPs or vehicle were given in the drinking water at a dose of 100 mg/kg/day for the following 26 days. At the end of the treatment period, we investigated the macrovessel density in tumors by high definition microCT system using radio opaque silicon rubber and the microvessel density by immunohistochemistry (anti-CD31) on frozen sections. In parallel, we measured an index of proliferation (Ki-67) and apoptosis (TUNEL, activated caspase-3) and we determined the expression level of pro-angiogenic factors (VEGF, MMPs) and tumor suppressor genes (*p21, p16, p53* and *p73*) on paraffin sections. *Results:* After one month of treatment with RWPs, tumor size was significantly reduced by 30% compared to the control group. On the one hand, the vessel density assessed by microCT and immunohistochemistry, was reduced by 40% and by 47%, respectively in the RWPs-treated group. We observed a concomitant decreased expression levels of the major pro-angiogenic factors VEGF and MMPs 2 and 9 (MMP-2,9) in tumor cells. On the other hand, RWP treatment reduced the proliferating index (Ki-67) of tumor cells by 61% and increased the apoptotic index (TUNEL) associated with high caspase-3 activity in tumor cells by 81%. Finally, RWPs also induce the expression of tumor suppressor genes such as *p16, p21, p53* and *p73* in tumor cells. *Conclusion:* RWPs reduce tumor growth by preventing tumor neovascularisation and by inducing tumor cell apoptosis through the expression of tumor suppressor genes. Altogether, these findings indicate that RWPs have potent anticancer properties. As this syngenic model is very remote from the human physiopathology, further studies are necessary to highlight the anticancer properties of RWPs in a more relevant model of colon cancer.

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PRECANCEROUS CARCINOGENESIS–CELLULAR MODEL OF HUMAN BREAST EPITHELIAL CELLS FOR DIETARY PREVENTION

Hwa-Chain Robert Wang

Department of Pathobiology, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN 37996, USA

Carcinogenesis of human breast epithelial cells from non-cancerous to precancerous and cancerous stages is a multiyear, multistep, and multipath disease process. Exposure to environmental carcinogens has been postulated to increase the risk of developing human breast cancer. However, the role of environmental carcinogens in the chronic carcinogenesis of human breast epithelia has not been fully addressed. Conversely, epidemiological and experimental evidence suggests that dietary constituents in fruits and vegetables prevent human cancer. Still, the mechanisms for dietary prevention of human breast cells from precancerous carcinogenesis induced by chronic exposure to environmental carcinogens remain to be elucidated. To investigate dietary prevention of cellular carcinogenesis, we studied potential preventative biological, biochemical, and transcriptomic target endpoints induced by long-term exposure of human breast epithelial cells to environmental pollutions with low doses of carcinogens. Immortalized, non-cancerous human breast epithelial MCF10A cells were repeatedly exposed to combined environmental carcinogens, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[a]pyrene (B[a]P), each at picomolar concentrations, resulting in the induction of an increased acquisition of cancer-related biological, biochemical, and transcriptomic changes as target endpoints. However, long-term exposure to these carcinogens did not induce cellular tumorigenicity. Accordingly, our carcinogenesis-cellular system mimics chronic carcinogenesis of breast cells to progressively produce precancerous cells in an accumulative manner as it occurs at the precancerous stage, ductal carcinoma *in situ*, of human breast cancer. We then used this precancerous model to reveal the ability of dietary grape seed proanthocyanidin and green tea catechins to block acquisition of target endpoints in the suppression of cellular carcinogenesis. Thus, our precancerous carcinogenesis-cellular model can serve as a cost-efficient, *in vitro* system to identify bioactive dietary components that are able to suppress human breast cell carcinogenesis associated with long-term exposure to carcinogens. This research is expected to benefit society by identifying molecular targets for bioactive dietary components to formulate dietary supplements that are able to reduce the health risk of cancer caused by long-term exposure to carcinogens in environmental pollution.

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ROLE OF REACTIVE OXYGEN SPECIES IN THE PRO-APOPTOTIC ABILITY OF ONCOGENIC H-RAS TO FACILITATE APOPTOSIS INDUCED BY HISTONE DEACETYLASE INHIBITORS

Hwa-Chain Robert Wang

Department of Pathobiology, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN 37996, USA

More than 35% of human urinary bladder cancer involves oncogenic H-Ras activation. Expression of oncogenic H-Ras not only promotes tumorigenesis of human urinary bladder cancer cells, but also increases cellular susceptibility to histone deacetylase inhibitors (HDACI), such as FK228 (a depsipeptide also known as FR901228 and romidepsin) and trichostatin A, for inducing selective apoptosis. The novel pro-apoptotic ability of oncogenic H-Ras should be seriously considered in therapeutic designs for controlling oncogenic H-Ras-involved cancer. However, the mechanisms behind the proapoptotic ability of oncogenic H-Ras to enhance cell susceptibility to HDACI remain unclear. Our recent studies revealed that ectopic expression of oncogenic H-Ras increased intracellular reactive oxygen species (ROS) in human urinary bladder cancer J82 cells, enhanced FK228-increased intracellular ROS production from mitochondria, and increased cell susceptibility to exogenous ROS for inducing apoptosis and caspases. The extrinsic caspase-8 and intrinsic caspase-9 pathways were sequentially induced by FK228-increased ROS to cooperatively induce a profound activation of the executioner caspase-3/7 for inducing selective apoptosis of oncogenic H-Ras-expressing cells *versus* parental cells. Although FasL expression was induced by FR901228 in J82 cells in an ROS-dependent manner, the ROS-dependent activation of caspase pathways and cell death were induced by FR901228 in a FasL-independent manner. Our results led us to a suggestion that increased ROS played a contributing role in the proapoptotic ability of oncogenic H-Ras to enhance FK228-induced activation of caspases for inducing selective apoptosis in a dose-dependent manner.

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DOWN-REGULATION OF GRP78 IS ASSOCIATED WITH THE SENSITIVITY OF CHEMOTHERAPY TO VP-16 IN SMALL CELL LUNG CANCER NCI-H446 CELLS

Yingyan Wang[1], Wei Wang[2], Jiarui Wang[3] and Qi Wang[4]

[1]Diagnostics Laboratory Center, [3]Postgraduate School, Dalian Medical University, [2]Department of Respiratory Medicine, The Third People's Hospital of Dalian, [4]Department of Respiratory Medicine, The Second Hospital Affiliated to Dalian Medical University, Dalian, Liaoning, 116023, PR China

Background: Lung cancer is currently the leading cause of cancer deaths worldwide. Small cell lung cancer (SCLC) is the main type of lung cancer. Chemotherapy is an important means of treatment for SCLC. However, the treatment is limited by the development of drug resistance. An important mechanism of chemotherapy resistance is the synthesis of a kind of evolutionarily conserved proteins, named as glucose-

regulated proteins (GRPs). GRP78/BiP, a well-characterized GRP member with molecular weight of 78 kda, can be down or up-regulated by some agents such as inhibitors of Ca^{2+} -chelator BAPTA-AM or inducers of calcium ionophore A23187. Many reports have shown that GRP78 plays a protective role in maintaining cell viability against several kinds of stress in a variety of cancers. However, most of the reports focus on the up-regulation of GRP78, whereas only few of them examine the down-regulation of GRP78, especially whether the suppression of GRP78 is associated with the sensitivity of chemotherapy in cancer. Herein, we intended to investigate the down-regulation of GRP78 by the inhibitor of BAPTA-AM and the function of this inhibition in the resistance to VP-16 in SCLC NCI-H446 cells. *Methods:* NCI-H446 cells were divided into three groups: BAPTA-AM-treated group, A23187-treated group and control-group. Immunofluorescence and RT-PCR were used to assess the expression of GRP78 at both protein and mRNA levels for the three cell groups. Then the cells were treated with VP-16 at the concentration of 30 μM, and percentages of apoptosis and the cycle distributions of the cells were detected by flow cytometry. *Results:* Compared with A23187-treated and control group cells, the expression of GRP78 at both protein and mRNA levels for the BAPTA-AM- treated cells were greatly decreased in a dose-depended manner. After treatment with VP-16, the percentage of apoptotic cells for BAPTA-AM-treated group were: 33.4±1.01%, 48.2±1.77%, 53.0±1.43%, 56.5±2.13%, respectively, corresponding to the concentrations of BAPTA-AM 10, 15, 25, 40 μM, which was statistically significant high in comparison with the A23187-treated group and untreated-group (7.18±1.03 % and 27.8±1.45%, respectively, $p<0.05$). The cell cycle distributions assayed by flow cytometry showed that there was a great decrease in G1 phase and a dramatic increase in S phase for the BAPTA-AM-treated cells compared with the untreated cells. *Conclusion:* BAPTA-AM is a strong inhibitor of GRP78 in NCI-H446 cell line. This suppression of GRP78 is significantly associated with the sensitivity to VP-16 in NCI-H446 cells through the alteration of cell cycle distributions. These results indicate that down-regulation of GRP78 or the inhibition of GRP78 activity may offer a new therapeutic approach to the clinical management of lung cancer.

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CONSTRUCTION OF AN ARTIFICIAL RADIATION-GUIDED SYNCHRONIZING GENE CIRCUIT USING NITRIC OXIDE SIGNALING PATHWAY IN HUMAN LUNG CANCER CELLS

Wei-dong Wang, Zheng-tang Chen and De-zhi Li

Cancer Center of PLA, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China

Aim: Radio-genetic therapy is a novel strategy for cancer treatment, however, the expression level of therapeutic genes induced by radiation is too low to completely eradicate the tumor, especially under the routine clinical doses of radiation. Gene circuits are some simple gene networks characterized by special regulatory properties. *Methods:* In the present study, a synchronous amplifying gene circuit controlled by nitric oxide (NO) signaling elements was generated by coupling c-fos promoter with inducible nitric oxide synthase (iNOS), which is induced by ionizing radiation owing to the radiation-responsive of c-fos promoter. *Results:* The synchronous amplifying property of the constructed gene circuit was demonstrated by assaying of green fluorescence protein with three-fold enhanced expression. In a further study, the suicide gene *HSV-TK* substituted the reporter gene; the duration and level of expression of target genes increased and the enhanced radiosensitivity and therapeutic effects on human lung cancer were also shown in this experiment. *Conclusion:* The study will improve the radio-genetic therapy in future by promoting the expression level and prolonging the duration of expression of target genes.

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CHROMATIN RESTORATION FOLLOWING GENOMIC REPAIR

Qianzheng Zhu, Gulzar Wani, Hany Arab, Mohamed A. El-Mahdy, Alo Ray and Altaf A. Wani

Department of Radiology, The Ohio State University Medical Center, Columbus, OH 43210, USA

DNA damage provokes activation of cell cycle checkpoint response allowing time for DNA repair to maintain genomic integrity. During DNA damage response and repair, the chromatin is transiently disrupted to overcome the structural barrier and provide repair machinery access to damaged sites. Thus, restoration of functionally intact chromatin structure following DNA damage processing is crucial for retaining genetic as well as epigenetic information coded within modified histones. Our recent research has delved into how chromosomal incorporation of ubiquitinated histone H2A (uH2A) occurs following damage repair and the characteristic of this process in the context of nucleotide excision repair (NER) sub-pathways of global genomic repair (GGR) and transcription-coupled repair (TCR).

We demonstrate that UV-induced uH2A foci formation occurs at cyclobutane pyrimidine dimer (CPD) sites in cells lacking XPC, DDB2, CSA or CSB, but not in cells lacking XPA, XPG or XPF. This repair-proficient and -deficient cell response indicated that uH2A incorporation strictly relies on successful damage repair occurring through either GGR

or TCR sub-pathway. In contrast, XPA, XPG or XPF were not required for the formation of γH2AX foci in asynchronous cells, affirming this event as a pre-incision process. Notably, the H2A ubiquitin ligase Ring1B, a component of Polycomb repressor complex 1, did not localize at DNA damage sites. However, histone chaperone Caf-1 showed distinct localization to the damage sites. Knockdown of Caf-1 p60 abolished Caf-1 as well as uH2A foci formation. Caf-1 p150 was found to associate with NER factors TFIIH, RPA p70 and PCNA in chromatin. Overall data demonstrate that prompt Caf-1-mediated chromatin restoration following NER puts uH2A marks at the sites of repaired damage within chromatin. Our observations on CPD repair, and recent report on acetylated histone H3 driving the chromatin assembly after DSB repair, unfolds a novel phenomenon related to DNA repair-mediated alterations of epigenetic information within chromatin.

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HD-PTP IS IMPORTANT REGULATOR OF PDGFRβ DEGRADATION

Piotr Wardega and Johan Lennartsson

Ludwig Institute for Cancer Research, Uppsala University, Box 595, SE-751 24 Uppsala, Sweden

Human His-domain-containing protein tyrosine phosphatase (HD-PTP) is a novel protein that has been implicated in sorting of internalized proteins towards degradation, although the molecular mechanism remain unclear. Previous studies have suggested that HD-PTP may interact with several components sorting machinery such as TSG101 (member of endosomal sorting compexes required for transport- I, ESCRT-I), CHMP4 (forms ESCR-III complex together with other proteins), endophilin A1, but not with CIN85. We have investigated the role of HD-PTP in down-regulation and degradation of platelet-derived growth factor receptor β (PDGFRβ). Our data indicate that HD-PTP is able to interact in a constitutive manner with PDGFRβ. We observed that HD-PTP is tyrosine phosphorylated under steady-state conditions, but this phosphorylation can be further increased by PDGF-BB stimulation. Elevated expression of HD-PTP increased PDGF-induced tyrosine phosphorylation of PDGFRβ, but decreased receptor ubiquitination. Furthermore, we observed that HD-PTP overexpression reduced the level of c-Cbl phosphorylation, consistent with the decreased level of PDGFRβ ubiquitination. In conclusion, our findings suggest that HD-PTP interferes with c-Cbl's ability to induce PDGF dependent receptor ubiquitination and thereby the process of receptor down-regulation.

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PREDICTION OF RESPONSE IN MULTIMODALITY TREATMENT OF UPPER GASTROINTESTINAL CANCER: IMPACT OF MOLECULAR MARKERS

Ute Warnecke-Eberz[1], Elfriede Bollschweiler[1], Uta Drebber[2], Lukas Heukamp[3], Stephan E. Baldus[4], Hakan Alakus[1], Daniel Vallböhmer[1], Paul M. Schneider[5], Arnulf H. Hoelscher[1] and Ralf Metzger[1]

[1]Department of General, Visceral and Cancer Surgery, [2]Institute of Pathology, [3]University of Cologne, Institute of Pathology, University of Bonn, [4]Institute of Pathology, Heinrich-Heine-University Duesseldorf, Germany; [5]Department of Visceral and Transplantation Surgery, University Hospital Zurich, Switzerland

Malignant tumors arise when cells change their developmental program and replicate independently from signal cascades and cellular control mechanisms. The malignant development is genetically directed. The aim of our work was to exploit these genetical changes for diagnosis, response prediction and monitoring for clinical purposes. Since only 30 to 50% of patients with locally advanced esophageal cancer (cT2-4, Nx, M0) benefit from neoadjuvant radiochemotherapy (cisplatin, 5-FU, 36 Gy) there is a need for predictive markers.

The predictive impact of a selection of genes was analyzed by real-time RT-PCR quantification. Expression levels were compared with the degree of histopathological tumor regression. Tumor regression was defined as major response when resected specimens contained fewer than 10% residual vital tumor cells. The excision repair gene *ERCC1* is essential for removal of DNA damage, caused by radiation and chemotherapy. The predictive impact of *ERCC1* mRNA levels was demonstrated. An increased level of *ERCC1* mRNA in pre-therapeutical tumor biopsies was not compatible with a major response (sensitivity 62.5%, specificity 100%). A total of 42% of patients were identified for non-response to the neoadjuvant treatment by quantification of *ERCC1* mRNA. *c-erbB-2* mRNA expression proved to be an additional predictive marker of minor histopathological response to neoadjuvant therapy in patients with esophageal cancer.

Complementing gene expression analysis, single nucleotide polymorphisms proved to be associated with therapy response. By genetic variants of polymorphism rs11615, 38% of the patients were identified as minor responders and could be prevented from a non-effective neoadjuvant treatment. A minor group of 9.6% would miss a potentially successful therapy. XRCC1 is part of the base excision repair system (BER) repairing small lesions around the damaged base. The rarely appearing genotypes AA and AG of *XRCC1* polymorphism rs1799782 proved to be additional markers for response prediction. These data

confirm that DNA repair capacity is a critical mechanism for resistance to platin-based therapeutics.

Since analysis of single molecular markers allows response prediction only at a low sensitivity and specificity, co-expression of different candidate genes was applied. For prediction of therapy response in esophageal cancer, the predictive impact of a panel of 17 target genes was analyzed. Expression of mRNA in paired pretherapeutical tumor/normal biopsies was quantified by TaqMan Low Density Array (LDA) analysis. LDA proved to be a standardized and reproducible method for clinical practice. Combination of a gene panel consisting of *c-erbB-2, cdc25b, DPD, ERCC1, p21, p16, PAI-1* and *VEGF* increased the predictive accuracy. Construction of a predictive model by artificial neuronal network analysis for prediction of major histopathological response provided an accuracy of 90.5%, at a sensitivity of 80% and a specificity of 90.5%.

For identification of novel markers not yet associated with response prediction, human genome microarrays were applied. Verification experiments confirmed the response predictive impact of two genes: *Cullin 2*, a member of SCF E3 ubiquitin ligase complex, modifying proteins involved in cell cycle progression, and *STK11*, a serine/thereonine kinase tumor suppressor gene. An LDA consisting of an optimized gene panel could be applied to customize treatment strategies by quantification of gene expression patterns.

Regarding esophageal, lung and pancreatic cancer overexpression of *survivin*, an inhibitor of apoptosis, proved to be a marker for detection of cancer. In esophageal cancer, we were able to show that *survivin* is additionally a marker for monitoring of the therapy. Detection of *survivin* mRNA was also established for blood samples. *Survivin* mRNA was monitored in 88% of the patients with gastrointestinal tumors. A total of 59% of the patients showed a significantly lower median *survivin* mRNA expression after surgical resection than before. Following neoadjuvant therapy and tumor resection, in 38% of the patients there was no *survivin* mRNA detectable at all. The data confirm the impact of survivin mRNA expression for detection of dissiminating tumor cells in peripheral blood and for monitoring of the therapy.

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DEVELOPMENT AND APPLICATION OF A SPECIFIC MONOCLONAL ANTIBODY FOR THYROID CANCER

Michiko Watanabe[1], Hiroshi Takeyama[2] and Yoshinobu Manome[1]

[1]Department of Molecular Cell Biology, [2]Department of Surgery, Jikei University School of Medicine. 3-25-8 Nishishin-bashi, Minato-ku, Tokyo 104-8461, Japan

Thyroid cancer has been diagnosed conventionally by fine-needle aspiration (FNA). However, even though the diagnostic accuracy of FNA has increased, 20% of cases still require further investigation to determine if the lesion is benign or malignant. Other diagnostic procedures such as echography, scintigraphy, and CTscanning are of little help. Therefore, development of a more accurate system is required.

A monoclonal antibody, JT95, was established by Takeyama, Watanabe *et al*., which specifically reacts to human thyroid cancer. The immunohistochemical reactivity of JT95 was 96% to papillary carcinoma and 75% to follicular carcinoma, but it showed hardly any reaction to normal tissues. This specific reactivity was confirmed on 288 cases of thyroid cancer in 13 medical facilities. The efficacy was studied under the jurisdiction of the Japanese Society of Thyroid Surgery.

This antibody recognized a glycoprotein containing sialic acid and which had a molecular weight of 250 kDa. Amino-acid sequencing revealed that the antigen was glycosylated fibronectin. An approximately half-sized, 105-kDa tumor-related antigen was found to be circulating in the body of the patients and was detected in the blood by an immunoblot assay. In the serodiagnosis using an enzyme-linked immunosorbent assay, JT95 detected 80% of relapsed or metastasized thyroid cancer. In contrast, the detection rate was merely 51% in the primary patients. To improve the sensitivity and enable precise quantification, we are currently attempting to label the antibody with nano-particles. In addition, immunohistochemical investigation using the antibody contributes to the understanding of tumor antigen distribution and biological activities in thyroid diseases. Increased sensitivity of JT95 will raise the potential for use of JT95 in diagnosis and treatment. Monoclonal antibodies have become more important in both research and clinical applications. We consider that clinical use of the JT95 antibody might be another therapeutic application.

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FAMILIAL CANCER SUSCEPTIBILITY SYNDROMES HAVE DISTINCTIVE CLINICAL FEATURES

Walter Weber, Jacqueline Estoppey and Hansruedi Stoll

Medical Oncology, Heuberg 16, CH-4051 Basel, Switzerland

Introduction: Every human disease clusters in families to some extent. This is also true for cancer. An excess of cancers of the same site exists among relatives, with thyroid and colon cancers and lymphocytic leukaemia showing the highest familial risks (1). There is also familial clustering of malignancies originating from different primary sites (2). Primary care physicians approve syndrome-specific germline mutation testing and want to play a central role in the management of cancer families (3). Therefore, characteristics of hereditary cancer predisposition syndromes have to be known (4). *Methods:* In Basel

families with conspicuous cancer aggregations can be referred for work-up and counseling to medical oncology in the University Hospital or in a private practice nearby. *Results:* In 10 cancer families with germline mutations (4 breast-ovarian ca., 4 colorectal ca., one gastric ca. and one Cowden syndrome) and one renal cancer family with a balanced translocation (t(3;11) (q13,3;q21) the following distinctive features were observed: multiple primary tumors, multifocality, younger-than-usual age at tumor diagnosis, association with muco-cutaneous lesions (genodermatoses) and histological pecularities (rare histology, lobular breast cancer, mucinous adenocarcinomas of colorectum and diffuse type gastric cancer). *Conclusion:* Distinctive features of familial cancer susceptibility syndromes should be known in the light of growing evidence of the efficacy of medical and surgical interventions.

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DETECTION AND CHARACTERIZATION OF CTC IN CARCINOMAS: NEW INSIGHTS

Janine Wechsler[1] and Yvon Cayre[2]

[1]Department of Pathology, Henri Mondor University Hospital, Créteil and [2]Department of Clinical Hematology, Robert Debré University Hospital, Paris, France

Despite the advances in oncology, the mechanisms by which circulating tumor cells (CTC) are generated, and their involvement in the metastatic process are still under debate. Carcinomas representing 80% of all cancers are used as a model to elucidate the important question of systemic tumor cell dissemination. Although extremely rare, CTC represent a potential alternative to invasive biopsies as a source of tumor tissue. Several methods for detecting CTC in peripheral blood from patients with various carcinomas have been developed over the past few decades. Immunocytochemical and molecular assays now enable the specific detection of CTC even at the single-cell stage. In the present review, we provide a critical review of the current methods used for detection of CTC and data on their morphological and molecular characterization. In a variety of carcinomas including breast cancer, it is now well established that CTC counts predict overall survival and provide independent prognostic information to predict disease progression. The next challenge of CTC characterization will be to detect, in a non-invasive and serial manner, resistance/sensitivity of tumor cells to novel targeted therapies. Such a genetic-based analysis appears as a critical step in the development of a personalized medicine.

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INHIBITION OF SURVIVAL SIGNALING BY AN INDOLE-3-CARBINOL-DERIVED ANTITUMOR AGENT

Jing-Ru Weng[1,2,3], Yuan-Yin Chen[1], Chang-Fang Chiu[3], Ming-Hsui Tsai[3] and Ching-Shih Chen[4]

[1]Department of Biological Science and Technology, [2]Research Center for Biodiversity, China Medical University, Taiwan;
[3]Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung 404, Taiwan, ROC;
[4]Division of Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA

The chemopreventive potential of indole-3-carbinol has attracted much attention because of its demonstrated ability to protect against chemical-induced carcinogenesis in different experimental animal models. From a mechanistic perspective, the ability of indole-3-carbinol to target a broad range of signaling pathways underlies its translational potential for cancer prevention and/or treatment. However, its clinical use might be compromised by its complicated pharmacokinetic behaviors, poor bioavailability, and low antiproliferative potency *in vitro*, which render therapeutic concentrations difficult to achieve in the body. Therefore, this study was aimed at pharmacologically exploiting indole-3-carbinol as a molecular platform to develop structural variants with improved chemical stability and apoptosis-inducing potency. Among a series of indole-3-carbinol derivatives examined, OSU-A9 represented the optimal agent, with an IC_{50} of 3.8 μM and 2.0 μM for reducing the viability of SCC9 and SCC2095 human oral cancer cell lines, respectively. Cell viability, apoptosis and signaling targets were determined by MTT, ELISA, immunoblotting and cell counting. Despite a 100-fold difference in antitumor potency, the pharmacological profiles of OSU-A9 and indole-3-carbinol in interfering with target signaling pathways were virtually identical. OSU-A9 is a potent antitumor agent with pleotropic mechanisms of action by affecting multiple signaling pathways, which might have translational potential in oral cancer therapy.

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CELL SIGNALING AND CANCER-POSSIBLE TARGETS FOR THERAPY

C.E. Wenner

Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY. 14263, USA

Metabolic reprogramming in tumor cells involves acquisition of properties which include growth-factor independent cell proliferation, failure of inhibition by growth-inhibitory signals,

ability to invade surrounding tissues, and to evade apoptosis, *etc*. Characterization of the profile or molecular signature of the tumor facilitates the development of rational therapies that target the aberrant pathways. Rapidly growing tumor cells are usually associated with high rates of glycolysis and in these cells, it appears advantageous to exploit this pathway which most likely is required for optimal synthetic needs. Glycolysis is regulated at several control points and the association of hexokinase with mitochondria is observed with rapidly growing, highly glycolytic cells. Hexokinase II (HK-II) is overexpressed in a number of rapidly growing tumors and binds to the outer mitochondrial membrane. Phosphorylation of Voltage–Dependent Anion Channel (VDAC) plays a role in the regulation of HK-II binding (Pastorino-J Bioenerg Biomembr, 2008) The disruption of hexokinase/ mitochondrial interaction impairs anti-apoptotic and mitochondrial integrity-promoting functions of PKB/Akt. It is our view that the metabolic regulatory functions of PKB/Akt have evolved into an adaptive sensing system involving mitochondrial hexokinases. Therefore, targeting HK enzyme activity and its association with integral membrane components such as VDAC offers potential therapeutic benefit. Recent studiesl by Chiara *et al*. (Plos One *3*: 1852, 2008) demonstrated that Hexokinase II detachment from mitochondria triggers apoptosis through the permeability transition pore but independent of VDAC. Although these results imply that VDAC–hexokinase interaction are not irreplaceable constituents of the permeabilization process, it is possible that alternate membrane constituents can act as binding partners for the HK II in the absence of VDAC. Alternate constituents could also be capable of opening of the permeability transition pore. The studies of Chiara *et al*. contribute greatly to our understanding of the pathways of outer mitochondrial membrane permeabilization and their inactivation of tumors. For example, a hexokinase-II N-terminal peptide selectively detaches HK-II from mitochondria and induces apoptosis. The development of highly specific inhibitors in conjunction with combinatorial therapeutic agents which target the growth factor signal transduction pathways as well as apoptotic signaling pathways should provide an opportunity for maximal therapeutic benefit.

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ANTICANCER PROPERTIES AND MULTIDRUG RESISTANCE MODULATORY EFFECT OF PLANT POLYPHENOLS AND THEIR INTERACTION WITH MEMBRANE COMPONENTS

Krystyna Michalak[1], Olga Wesołowska[1], Jerzy Wiśniewski[1], Kamila Środa[1], Andrzej Teisseyre[1], Michał Kużdżał[1], Janez Štrancar[2], Noélia Duarte[3] and Maria-José U. Ferreira[3]

[1]Department of Biophysics, Wrocław Medical University, ul. Chałubińskiego 10, 50368 Wrocław, Poland;
[2]Laboratory of Biophysics, Jozef Stefan Institute, Jamova 39, Ljubljana, Slovenia;
[3]CECF/iMed. UL, Faculty of Pharmacy, University of Lisbon, Av. das Forças Armadas, 1600-083 Lisbon, Portugal

Many compounds of plant origin were formed to be interesting for potential application in cancer prevention or chemotherapy. Such agents were found among flavonoids and stilbenes. In broad *in vitro* and *in vivo* studies, the ability of plant-derived polyphenols to interact with different cellular targets was revealed. Resveratrol (*trans*-3,4,5'- trihydroxystilbene) influences many cellular signaling pathways and affects all three stages of carcinogenesis. In this work the effect of several stilbenes (resveratrol, its analogue piceatannol, and piceatannol derivatives) and some flavonoids on the activity of ATP-binding cassette (ABC) multidrug transporters and potassium channels Kv1.3 was examined. Recently the role of potassium channel activity in cancer has emerged. Several of the compounds studied were potent potassium channel inhibitors. Strong inhibition of membrane transporters and ion channels was often observed in case of hydroxy-, methoxy-, acetoxy- and prenyl-derivatives in relation to the parent compounds. Piceatannol, naringenin and some of their derivatives occurred to be the most active inhibitors of MRP1 multidrug transporter. The influence of plant polyphenols on integral membrane proteins such as MDR transporters and ion channels can be mediated at least in part by non-specific membrane effects. Flavonoids and stilbene interactions with lipid bilayers were determined by fluorescence and ESR spectroscopy. Simulation of the experimental ESR spectra and application of GHOST condensation method were applied to study of the effect of polyphenols on membrane domain structure. The significance of the interaction of studied phytochemicals with membrane transporters, channels and lipid bilayer for their anticancer properties was discussed

Antiproliferative properties of the compounds were tested in doxorubicin-sensitive and -resistant, P-gp overexpressing colon cancer cell lines LoVo and LoVo/Dx, respectively. Tangeretin, natural polymethoxylated flavone and 8-prenylnaringenin most effectively inhibited cell growth both in sensitive and resistant cancer cell lines. Antiproliferative action was compared with pro-apoptic properties of the compounds.

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8-PRENYLNARINGENIN INHIBITS BOTH P-GLYCOPROTEIN AND MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 1

Olga Wesołowska[1], Jerzy Wiśniewski[1], Maria Paprocka[2], Danuta Duś[2] and Krystyna Michalak[1]

[1]Department of Biophysics, Wrocław Medical University, ul. Chałubińskiego 10, 50368 Wrocław;

[2]Laboratory of Cellular Interactions, Department of Medical Immunology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, ul. Weigla 12, 53-114 Wrocław, Poland

8-Prenylnaringenin (8-isopentenylnaringenin) is a potent phytoestrogen isolated from hop (*Humulus lupulus*). Apart from binding to estrogen receptors α and β this flavonoid was also recognized as an inhibitor of angiogenesis and a chemopreventive agent. In the present work we have studied the interaction of 8-prenylnaringenin with two multidrug-resistance-associated transporters, P-glycoprotein and MRP1. Functional test based on the transport of fluorescent substrate BCECF revealed that the flavonoid significantly inhibited MRP1 transport activity in human erythrocytes (IC_{50}=5.76±1.80 μM). The influence of 8-prenylnaringenin on P-glycoprotein was investigated in two human colon cancer cell lines, drug-sensitive LoVo and doxorubicin-resistant LoVo/Dx. By means of flow cytometry it was shown that the studied flavonoid significantly increased the accumulation of rhodamine 123 in drug-resistant cancer cell line. The ability of 8-prenylnaringenin to inhibit P-glycoprotein was also demonstrated by confocal microscopy. Sulforhodamine B (SRB) assay was employed to study the cytotoxicity of 8-prenylnaringenin. It was found that the flavonoid was more toxic to LoVo than to LoVo/Dx cell line, however in concentrations higher than 25 μM its toxicity to both cell lines was considerable. In conclusion, 8-prenylnaringenin was identified as a new potent multidrug resistance modulator. The flavonoid effectively inhibited two main MDR transporters: P-glycoprotein and MRP1 in concentrations that were not seriously toxic to the cells. In concentrations above 50 μM 8-prenylnaringenin acted as an anticancer agent, effectively killing the cancer cells.

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HOST POLYMORPHISMS AS RISK FACTORS FOR GASTRIC CANCER

Thomas Wex and Peter Malfertheiner

Clinic of Gastroenterology, Hepatology and Infectious Diseases, Leipziger Str. 44, D-39120 Magdeburg, Germany

Infection of the stomach with the gram-negative bacterium *Helicobacter pylori* is the main risk factor for the development of gastric cancer (GC) leading to its classification as a "definite carcinogen" by the World Health Organization in 1994. Since then, the pathophysiological mechanisms linked to this infection have been studied extensively in clinical and basic science. The current paradigm of gastric carcinogenesis is based on a multifactorial risk factors, the virulence factors of the bacterium (*e.g.* CagA, VacA), environmental factors (diet, smoking) and host factors (gene polymorphisms). The complex interplay among these factors determines the clinical outcome of the infection, leading to 3 major diseases in 1 out of 7 infected persons, namely ulcer disease, GC and mucosa-associated lymphoid tissue lymphoma in 15%, 1% and 0.1% of all persons infected with *H. pylori*, respectively.

In 2000, El-Omar and co-workers identified a gene polymorphism of the interleukin (IL)-1 gene that is associated with an increased risk for gastric cancer and shed light into the pathophysiology of the *H. pylori* infection and its molecular links to gastric carcinogenesis. In recent years, this work has been tremendously extended and various genomic variants, carrying a risk for the development of gastric cancer, were identified. Among them are cytokine genes (IL-8, IL-10) and receptors for the recognition of the bacterium (TLR-4). Besides the identification of novel gene polymorphisms linked to GC, it has become clear that there is no general genomic risk pattern for all GC patients. Most of the established host factors are restricted either to the histological type (intestinal *vs.* diffuse), ethnical background (Caucasian *vs.* Asian) on tumor localization (non-cardia *vs.* proximal GC). Here, we will review the current knowledge of the role of these host factors for gastric carcinogenesis and present our data concerning the role of *IL-1beta* and *NOD-2* gene polymorphisms in German patients with gastric cancer.

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kPCR-BASED IDENTIFICATION OF LOW-RISK AND HIGH-RISK BREAST CANCER PATIENTS FOR ALTERNATIVE TREATMENT OPTIONS

Ralph M. Wirtz, Mathias Gehrmann, Udo Stropp, Guido Hennig, Christian von Törne, Karsten Weber, Ralf Kronenwett and Christoph Petry

Siemens Healthcare Diagnostics; Molecular Research Germany; Nattermann Allee 1, 50829 Köln, Germany

Genomic approaches have revolutionized our understanding of tumor biology. Employing microarray analysis of fresh tissue tumor samples in a groundbreaking study Perou and co-workers (1) were first to identify subtypes intrinsic to breast cancer. Importantly, these molecular subtypes do not only differ fundamentally in their general aggressiveness, but also in their responsiveness to different anticancer therapies. However, tests to define these intrinsic subtypes have not yet been adapted to formalin-fixed tumor tissue, an obstacle to their use in routine clinical practice.

At Siemens Healthcare Diagnostics, we have analyzed gene expression and genomic alterations were examined in fresh and fixed tumor tissue samples of thousands of breast cancer

patients treated as part of clinical trials or in clinical practice. First, we identified a gene signature, consisting of twelve genes, that identifies node-negative breast cancer patients having a low-risk of developing distant metastasis within 5 years. The respective breast cancer prognosis score reached a sensitivity of 90% and a specificity of 35% for death after recurrence within 10 years in an independent validation cohort of 236 patients. The breast cancer prognosis score can be assessed by kPCR in formalin-fixed tumor tissue and therefore could be implemented into routine clinical practice.* This may help to spare low-risk breast cancer patients from harsh systemic treatments such as chemotherapy.

Second, we identified a gene signature consisting of only 4 genes that identifies high-risk patients in the node-negative and node-positive situation (>60% recurrence within 3 years) by molecular classification. Again, this gene signature was identified in fresh tissue specimens and then transferred to kPCR detection in formalin-fixed tissue samples. Importantly, this 4 gene algorithm not only identified the high-risk patient population in the validation cohort ($p<0.0001$; n=210), but also discriminated between low risk and high risk patients in the triple negative breast cancer subtype ($p=0.006$). As these patients were already treated with anthracycline and taxol-containing chemotherapy, alternative regimens might be applied to these high risk patients early on. Interestingly, the 4 gene algorithm comprises the detection of angiogenic activities in tumors and therefore might be helpful for selecting patients for antiangiogenic treatment options.

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THE TUMOR MICROENVIRONMENT: CONCEPTS AND GENERAL CHARACTERISTICS

Isaac P. Witz

Department o Cell Research and Immunology, Tel Aviv University, Tel Aviv, 69978, Israel

The tumor tissue is composed of 2 compartments intimately associated with each other. The first compartment constitutes the malignant cells. The second is the tumor microenvironment. This compartment is composed of resident cells such as fibroblasts, endothelial cells and other non-malignant cells; of infiltrating cells such as lymphocytes or macrophages and of numerous molecules such as those of the extracellular matrix, growth factors, cytokines, chemokines, antibodies, proteases, other types of enzymes and various metabolites. All these molecules may be released from the tumor cells and/or from the non malignant cells. Some artificially administered molecules, for example drugs, may also be present in the microenvironment. The microenvironment of many solid tumors may be characterized by hypoxia; low extracellular pH and by low glucose concentration.

The contemporary concept of tumor microenvironment postulates that it functions as an active "educational/inductive/selection" venue in which the tumor is directed into one of several molecular evolution pathways by microenvironmental factors. However the interaction between microenvironmental components and tumor cells is bidirectional. In addition to the regulation of genes in tumor cells by microenvironmental factors, tumor cells and their products are capable of regulating gene expression in non-tumor cells residing in or infiltrating into the microenvironment thereby altering their phenotype. There are scores of tumor-microenvironment interactions that play anti or pro malignancy roles. These include interactions that lead to or drive cell proliferation or death, angiogenesis, motility, chemotaxis, invasion, protective immunity, inflammation and metastasis to name a few. Many such interactions await their discovery and the significance of other interactions has still to be elucidated.

Interactions of cancer cells with components of their microenvironment are pivotal determinants in the decision making process that determines if cancer cells will progress towards metastasis or whether they will stay dormant or disappear altogether. The realization that metastasis is controlled by interactions of tumor cells with microenvironmental components has allowed for the establishment of a new paradigm, namely intervention in the metastatic process by targeting interactions between the tumor cell and its microenvironment. Numerous preclinical and clinical trials attempt, on the one hand, to block tumor-microenvironment interactions that boost tumor progression and metastasis and on the other hand enhance interactions that counteract malignancy.

In order to develop effective anti metastasis therapies and in view of the complexity of tumor-microenvironment interactions, combinatorial approaches used in the analysis of hyper complex systems will have to be employed.

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THE DYNAMIC SYSTEM [TELOMERES-TELOMERASE-PROLIFERATION] UNDERLIES PERENNIAL GROWTH OF CANCER CELLS AND EPISODES OF DRUG RESISTANCE

J. Deschatrette and C. Wolfrom

CNRS UMR8080 "Développement et évolution" Université Paris-Sud. 91405 Orsay, France

For a long time, regular progressive growth and resistance to anticancer drugs have been considered as invariant characteristics of a tumor. However, there are indications that both cell growth and drug resistance can display discontinuous patterns over months, although the mechanisms underlying these fluctuations are unclear. Our hypothesis is that telomere regulation plays an important role in such fluctuations. In rat hepatocarcinoma cells *in vitro*, we observed that every part of the small complex system [Telomeres-Telomerase-Proliferation] displayed deterministic oscillations. These oscillations were coordinated and resulted in an oscillatory conservative behavior of the whole system. This global dynamic equilibrium was found critical for perennial growth of hepatocarcinoma cells and for peaks of cell resistance to methotrexate.

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PHYSICAL ACTIVITY AND CANCER

Alicja Wolk

Karolinska Institutet, Department of Environmental Medicine, Division of Nutritional Epidemiology, Sweden

Physical activity promotes health, wellbeing and longevity through decreased risk for cardiovascular disease, cancer, other chronic diseases and all, as well as that for cancer, mortality.

Accumulating evidence from epidemiological studies shows that physical activity is of significance for reducing cancer incidence. The most consistent associations between physical activity and reduced cancer risk have been observed for colon and breast cancer. Growing evidence also indicates a reduced risk for endometrial and lung cancer. There has not been clear support for an inverse association between physical activity and prostate cancer risk in previous studies; however, more specific new analyses indicate that such an association may exist. Furthermore, growing evidence supports the role of physical activity in improving cancer prognosis and quality of life in cancer survivors. Less evidence is available on cancer recurrence.

Current recommendations regarding a required "dose" of daily physical activity in prevention of cancer development and cancer mortality are specified as 30-60 minutes per day of moderately-to-vigorously intense physical activity. They are further supported by new results from prospective studies of men and women in Sweden.

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***HELICOBACTER PYLORI* ERADICATION IN THE PREVENTION OF GASTRIC CANCER**

Benjamin C. Y. Wong

Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong

Gastric cancer remains one of the top cancer killers in the World. Chronic *Helicobacter pylori* infection increases the risk of gastric cancer, by stepwise progression from chronic active gastritis to gastric atrophy, intestinal metaplasia, dysplasia and finally cancer. These stepwise progressions may take many years, and at present there is no proven effective treatment for the presence of premalignant lesions including intestinal metaplasia or dysplasia. Hence the two prevailing questions in gastric cancer prevention are (a) whether treatment of *H. pylori*-related gastritis can reduce the risk of gastric cancer, and (b) whether treatment of *H. pylori*-related IM or dysplasia can both reverse the premalignant lesions and reduce the risk of gastric cancer.

Our randomized placebo controlled trial in China started in year 1994 included both patients with *H. pylori* related gastritis and patients with *H. pylori* related premalignant lesions (1). After 7.5 years of follow up, patients receiving *H. pylori* treatment showed a non-significant trend of having less gastric cancer than those patients that received placebo. The sub-group analysis showed that patients with *H. pylori* related gastritis benefited most from treatment, with no cancer developing in 7.5 years. However, in patients with *H. pylori* related premalignant lesions, there was no difference in the risk of gastric cancer in both treatment and placebo groups. Hence our study suggests that the benefit of treating *H. pylori* in cancer prevention may be restricted to patients with gastritis only.

Correa et al. performed another randomized placebo controlled trial in Columbia which included mainly patients with *H. pylori*- related premalignant lesions (2). Their 12-year follow up result suggested that subjects who were *H. pylori* negative after treatment had 14.8% more regression and 13.7% less progression than patients who were positive at 12 years (p=0.001). Hence he concludes that it is beneficial to treat *H. pylori* in patients with premalignant lesions. However the magnitude of benefit may be in the range of 15% only.

Based on these and other studies, the recommendation is that treatment of *H. pylori* is beneficial in prevention of gastric cancer. The benefit is greater in patients without premalignant lesions. Hence treatment earlier in life may give better results.

1 Wong BCY, Lam SK, Wong WM *et al*: *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region in China: A randomized controlled trial. JAMA *291(2)*: 187-194, 2004.

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SIMVASTATIN INDUCTION OF BCL-2 EXPRESSION AND NEUROPROTECTION IN MOUSE PRIMARY

NEURONS AND HUMAN NEUROBLASTOMA CELLS INDEPENDENT OF THE MEVALONATE/CHOLESTEROL PATHWAY

W. Gibson Wood

Department of Pharmacology, University of Minnesota School of Medicine, Minneapolis, Minnesota, USA

There are data, albeit with some controversy, indicating that the cholesterol lowering drugs, statins, may prevent certain types of cancer, are proapoptotic and may arrest cell growth. However, we and others have shown that statins afford protection to cells under various treatment conditions. Moreover, this protection is due in part to upregulation of Bcl-2. Simvastatin stimulated murine neuronal *Bcl-2* gene expression and protein levels *in vivo* and *in vitro*. Simvastatin pretreatment resulted in a significant reduction in cytotoxicity (caspase-3 activation, lactate dehydrogenate release) following a challenge with amyloid beta-protein compared with unchallenged neurons. G3139, an antisense oligonucleotide directed against Bcl-2, abolished the protective effects of simvastatin and eliminated simvastatin-induced up-regulation of Bcl-2 protein. Effects of simvastatin on Bcl-2 stimulation not only occurred in mice but were replicated in another species, the guinea pig. We demonstrated up-regulation of Bcl-2 in cerebral cortex of drug-treated guinea pigs and dissociated brain cells from simvastatin-treated guinea pigs were protected from neurotoxicity induced by sodium nitroprusside *ex vivo*. Simvastatin-induced neuroprotection was reduced by inactivation of the Bcl-2 protein, similar to effects observed when Bcl-2 protein levels were reduced by G3139. Effects of simvastatin on Bcl-2 stimulation were independent of inhibition of HMG-CoA reductase and data indicated the potential role of endothelin-1 on statin-induced stimulation of Bcl-2. Here, we provide novel evidence showing that simvastatin can stimulate expression of Bcl-2 both *in vivo* and *in vitro*. These findings provide one of the potential mechanisms for the purported neuroprotective effects of statins and do not support a proapoptotic mechanism of action of this class of drugs. Supported in part by NIH grants AG-18357 and AG-23524.

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BENZYL ISOTHIOCYANATE AND PHENETHYL ISOTHIOCYANATE INDUCE APOPTOSIS IN HUMAN OSTEOSARCOMA U-2 OS CELLS THROUGH THE PRODUCTION OF REACTIVE OXYGEN SPECIES AND MITOCHONDRIA- AND CASPASE-3-DEPENDENT PATHWAYS

Chang-Lin Wu and Jing-Gung Chung

Department of Biological Science and Technology, China Medical University, Taichung, Taiwan, ROC

From animal model studies, it has been shown that benzyl isothiocyanate (BITC) and phenethyl isothiocyanate (PEITC) inhibit carcinogenesis and tumorigenesis. However, the effects of BITC and PEITC on human osteosarcom U-2 OS cells are still not clear. In this study, we investigated whether or not BITC and PEITC can induce apoptosis in U-2 OS cells. U-2 OS cell proliferation was examined by MTT assay after exposure to BITC and PEITC *in vitro*. BITC at 7.5 μM and PEITC at 10 μM were used for different time treatments of U-2 OS cells for determination of cell viability. DAPI staining and comet assay were used for examining DNA damage and the results indicated that BITC and PEITC induced U-2 OS cell DNA damage in time- and dose-dependent manners. Flow cytometry was used for cell cycle analysis, sub-G1 group, production of reactive oxygen species (ROS) and Ca^{2+} and changes of mitochondrial membrane potential ($\Delta\Psi m$) in U-2 OS cells after treatment with BITC or PEITC for various time periods. BITC and PEITC reduced $\Delta\Psi m$ and increased the production of ROS and Ca^{2+} in U-2 OS cells in a time-dependent manner. BITC and PEITC induced G_2/M phase arrest in U-2 OS cells. In conclusion, BITC and PEITC promoted the production of ROS and Ca^{2+} and caused DNA damage and G_2/M phase arrest, reducing $\Delta\Psi m$ of mitochondria, resulting in activation of caspase-3 that finally led to apoptosis in U-2 OS cells.

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THE EFFECTS OF GAN-LUH-YIN ON THE PROLIFERATION OF LYMPHOCYTES AND SECRETIONS OF CYTOKINES FROM ORAL CANCER CELL LINES

Chia-Chun Wu[1], Jai-Sing Yang[2] and Jing-Gung Chung[1]

[1]School of Biological Science and Technology, [2]Department of Pharmacology, China Medical University, Taichung, Taiwain, ROC

Oral cancer is one of the major causes of death in the male population of Taiwan. It was reported that the number of T- and B-cells and the levels of interleukin (IL)-2 in oral cancer patients is lower then that of healthy individuals. However, the level of IL-4, IL-6 and tumor necrosis factor (TNF)-α in oral cancer patient is higher than that of healthy individuals. Gan-Luh-Yiin (GLY) is usually used for promoting cure by Chinese medicine in patients. Whether or not GLY can inhibit inflammation of oral cancer cells is not clear. Therefore, the purpose of our study was to investigate the proliferation of spleen cells from mice and also to examine the levels of cytokines from oral cancer cell lines such as CAL 27, SCC-4, HSC-3 and TW206. Celltiter kit was used for analysis of the total number of T- and B-cells. The CBA method and flow cytometry (FACS) were used to analyze 6

cytokines (IL-2, IL-4, IL-6, IL-10, TNF and interferon (IFN)-γ). The results indicate that GLY at 200, 500 and 1000 μg/ml promoted the proliferation of T- and B-cells under Con A and lipopolysaccharide stimulation, respectively. The results from CBA and FACS examinations indicated that: (1) GLY promoted the secretion of IL-2 from SCC-4 and TW206 cells; (2) GLY promoted the secretion of IL-4 from SCC-4, CAL 27, HSC-3 and TW206 cells; 3) GLY inhibited IL-6 secretion of SCC-4 cells; (4) GLY inhibited the secretion of TNF from CAL 27, SCC-4, HSC-3 and TW206 cells; and (5) it promoted the secretion of IFN-γ from CAL 27, SCC-4, HSC-3 and TW206 cells. In addition, GLY suppressed *TNF* mRNA level as examined by real-time PCR. GLY also suppressed the levels of proteins such as phosphorylated Akt, NF-κB p65 and ERK1/2 which were examined by Western blotting, NF-κB promoter assay and immunostaining in CAL 27 cells. Taken together, we suggest the effects of GLY on oral cancer may be mediated through anti-inflammatory action *via* TNF.

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MICRORNA ANALYSIS OF FORMALIN FIXED PARAFFIN–EMBEDDED BREAST CANCER TISSUES WITH DIFFERENT STAGES

Y. Zhao[1], C. Deng[2], H. Zhang[1], J. Xiao[1], R. Recker[1], Z. Gatalica[2], E. Silva[3] and G. Xiao[1]

[1]Genomics and Functional Proteomics Laboratories, Department of Medicine, Creighton University, Omaha, NE;
[2]Department of Pathology, Creighton University, Omaha, NE;
[3]Department of Surgery, Creighton University, Omaha, NE.

Introduction: Breast cancer still has the highest incidence among women in the United States. Our understanding of its molecular and cellular mechanisms is limited. Recently, microRNAs have gained favorable status as upstream regulators of breast cancer progression since then it can posttranscriptionally regulate sets of genes. It is now estimated that there are about 1000 miRNAs in the human genome, but only about 300 miRNAs have been identified in humans now. Much of miRNAs and their roles in cancer formation still await discovery. *Methods:* Formalin fixed paraffin–embedded (FFPE) breast tissues from different stages of the cancer were de-waxed before performing total RNA extraction. Tissues, from normal breast, *in situ* ductal carcinoma (DCIS), and invasive ductal carcinoma (IDC, stage II), with 15 subjects in each group were analyzed. The total RNA was extracted with acid-phenol: chloroform. MicroRNAs were isolated from total RNA. MicroRNA microarray profiling was performed using LC Sciences technology (LC Sciences, LLC). The Bioconductor implementation of Limma was used to analyze the data. *Results:* MicroRNA profiling experiments have been performed from 45 FFPE breast tissues of breast cancer subjects. By comparing the endogenous miRNA level in normal to that in DCIS, we found that numbers of miRNAs (*e.g.* mir-21) were significantly induced, while some of miRNAs (*e.g.* mir-205) were suppressed, in DCIS stage. In subjects with IDC stage, we observed some up-regulated miRNA species (*e.g.* mir-21) and more down-regulated miRNA species (*e.g.* mir-126), indicating that those miRNAs could be important candidate mediators that regulate progressive process of the breast cancer. These findings were confirmed by real time microRNA RT-PCR using breast cancer patient samples. Further functional studies are still under investigation.

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ASSOCIATION OF THE TOB ANTIPROLIFERATIVE PROTEIN WITH THE CCR4-NOT COMPLEX, A PLATFORM OF DEADENYLASE-BASED GENE REGULATION

Masahiro Morita, Kentaro Ito, Takashi Miyasaka, Toru Suzuki and Tadashi Yamamoto

The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan

The Tob/BTG family of antiproliferarive proteins is involved in multiple biological events that include cell growth suppression, apoptosis, and bone formation. To understand the underling mechanism by which Tob/BTG family proteins are involved in various biological events, we planned to identify Tob-interacting proteins. For this purpose, we established Flag-tagged Tob-expressing stable cell lines and applied a proteomic approach. We found that Tob interacts with the CCR4-NOT complex that is a multi-protein complex conserved from yeast to human. The human CCR4-NOT complex contains four enzymatic subunits, Cnot6, Cnot6L, Cnot7, and Cnot8, carrying the deadenylase activity, and at least five core and/or regulatory subunits: Cnot1 to -4 and Cnot9. We showed that the amino-terminal half of Tob interacts with Cnot7 and the carboxyl-terminal half interacts with Cnot1, a scaffold protein of the CCR4-NOT complex. Although we previously showed that Tob functions in the transcription machinery, the above finding suggests that Tob is also involved in the control of mRNA metabolism. We then addressed biological significance of the CCR4-NOT complex by depleting each component of the complex either by RNAi or targeted gene disruption in mice. Interestingly, we found that RNAi-mediated depletion of Cnot6L results in growth retardation of NIH 3T3 cells accompanied by elevation of both $p27^{Kip1}$ mRNA and $p27^{Kip1}$ protein. We showed that $p27^{Kip1}$ mRNA is stabilized and its poly (A) tail is preserved in Cnot6L-depleted cells. The data

suggest that Cnot6L regulates cell growth in a manner dependent on its deadenylase activity targeted to $p27^{Kip1}$ mRNA. We also found unique phenotypes in mice lacking Cnot3 and Cnot7, respectively. $Cnot7^{-/-}$ males are sterile owing to oligo-astheno-teratozoospermia, suggesting that Cnot7 deadenylase is essential for spermatogenesis. $Cnot3^{-/-}$ mice are embryonic lethal. Heterozygous mice for *Cnot3* are viable and fertile, but displayed severe leanness and hypersensitivity to insulin. We also showed Cnot3 positively regulates the CCR4-NOT-associated deadenylase activity. To clarify the underlying molecular mechanisms, we searched for the mRNA species whose abundance is altered in these gene manipulated mice. Taking all findings together, we will discuss the fundamental function of the CCR4-NOT complex in relation to the activity of Tob.

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DIFFERENTIAL PROGNOSTIC FACTORS INDEPENDENT OF TNM STAGE IN GASTRO-INTESTINAL CANCERS IN JAPAN

Keishi Yamashita, Akira Ooki, Hiroshi Katoh, MD, Mina Waraya and Masahiko Watanabe

The Department of Surgery, Kitasato University Hospital, Sagamihara, Kanagawa, Japan

Aim: To know the most important prognostic factors independent of TNM stage in advanced cancers such as esophageal, gastric, colorectal, and pancreatic cancer among the daily feasible clinicopathological factors. Materials: Advanced cancers of esophageal squamous cell carcinoma (ESCC); n=121, gastric adenocarcinoma (GAD); n=510, colorectal adenocarcinoma (CAD); n=596, pancreatic tubular adenocarcinoma (PAD); n=89. *Methods:* Univariate and multivariate analysis were used for prognostic relevance. TNM factors were excluded from the multivariate analysis in order to know the independent prognostic factors (IPF). *Results:* i) In ESCC, multivariate prognostic analysis revealed that lymph node metastasis density (ND)-factor and growth pattern were the strongest in more and less advanced ESCC, respectively. ii) In GAD, IDF was the ND-factor in stage II, III, and IV, reproducibly in both retrospective and prospective analysis. iii) On the other hand, in CAD, IDF was preoperative CA19-9 and multiple liver metastasis only in stage IV, and there were no other prognostic factors identified in curable cases. We will present diminishing prognostic impact of serum CEA in the recent patients. iv) In PAD, the strongest IDF was preoperative CA19-9, and the higher it was, the worse the patient prognosis was. Intra-operative dissected pancreatic margin (DPM) positivity was another IPF, and we could identify the long survivor group for advanced PAD. *Conclusion:* In ESCC and GAD, lymph node metastasis density (ND-factor) was the most life-threatening phenotype, while ND-factor in either CAD or PAD did not show such relevance for prognosis. On the other hand, preoperative CA19-9 was the most important prognostic factor in both CAD with stage IV and advanced PAD. We will describe the difference of such discrete types of cancers and present a putative mechanism to explain the difference. Therapeutic targets among the major gastro-intestinal cancers, will also be discussed.

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DYRK2 EXPRESSION CAN BE A PREDICTIVE MARKER FOR CHEMOTHERAPY IN NON-SMALL CELL LUNG CANCER

Shin-ichi Yamashita, Toshihiko Moroga, Kentaro Anami, Keita Tokuishi, Michiyo Miyawaki, Yozo Kawano, Masao Chujo, Satoshi Yamamoto and Katsunobu Kawahara

Department of Surgery II, Faculty of Medicine, Oita University, 1-1 Idaigaoka, Hasama, Yufu, Oita, 879-5593, Japan

Background: Several predictive markers of treatment and survival were reported such as ERCC1 in NSCLC (non-small-cell lung cancer). We report the possibility of DYRK2, a dual-specificity tyrosine-(Y)-phosphorylation regulated kinase gene to predict benefit from chemotherapy for patients with recurrent NSCLC. *Materials and Methods:* Forty patients with recurrent disease after surgery received several combinations of platinum-based chemotherapy. Chemotherapy effectiveness was evaluated according to RECIST criterion. We used immunohistochemical analysis to determine the expression of the DYRK2 protein with operative specimens of NSCLC. *Results:* We could not find any correlation between age, sex, pathological stage, tumor size, nodal status, histological type and DYRK2 expression. The overall response rate was 22.2% (4 out of 18) in DYRK2 positive group compared with 4.5% (1 out of 22) in negative group. On the other hand, the group of 17 PD (progressive disease) patients was consisted of 3 DYRK2 positive patients and 14 DYRK2 negative patients (p=0.0086). The median time to the progression of disease was 120 days in the DYRK2 negative group, as compared with 310 days in the DYRK2 positive group(HR=1.984, 95% CI=[1.039-3.788], p=0.034). *Conclusion:* Patients with recurrent NSCLC and DYRK2-positive tumors showed a substantial benefit from chemotherapy, as compared with patients with DYRK2-negative tumors.

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HMJ-30 SUPPRESSES MMP-2 EXPRESSION *VIA* RECEPTOR TYROSINE KINASE INHIBITION AND PKB/AKT, NF-KB, ERK SIGNALING PATHWAYS IN U2OS CELLS

Jai-Sing Yang[1], Yu-Jen Chiu[1], Kuan-Ting Chen[1], Mann-Jen Hour[2], Wen-Wen Huang[3] and Jing-Gung Chung[3]

[1]School of Medicine, [2]Graduate Institute and School of Pharmacy, [3]Department of Biological Science and Technology, China Medical University, Taichung, Taiwan, ROC

In this study, we examined the efficacy of HMJ-30, a quinazoline derivative, on cell metastasis of human osteosarcoma U2OS cells *in vitro* and explored its associated molecular mechanisms. The effects of HMJ-30 on U2OS cell viability, migration and invasion were detected by MTT, wound scratch assays and transwell invasion assays, respectively. Metastasis-relative protein expression and activity were determined by Western blotting and gelatin zymography. Our data showed that HMJ-30 inhibited U2OS cell proliferation, migration and invasion in a concentration-dependent manner. HMJ-30 inhibited protein expression of MMP-2 on HMJ-30-treated U2OS cells which were examined in Western blotting. HMJ-30 also suppressed MMP-2 activity in HMJ-30-treated U2OS cells as shown by gelatin zymography. HMJ-30 suppressed proteins of the receptor tyrosine kinase, including EGFR, ErbB2/neu and Met. HMJ-30 also suppressed Akt phosphorylation, NF-κB p65 and ERK1/2 protein expression in U20S cells. Taken together, our data indicate a possible role of HMJ-30 as a potential antitumor agent with the marked inhibition of metastatic and invasive capacity of human osteosarcoma U2OS cells.

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THE ROLE OF ENDOPLASMIC RETICULUM STRESS ON CELL CYCLE ARREST AND APOPTOSIS INDUCED BY CRUDE EXTRACT OF *EUPHORBIA FORMOSANA* RADIX IN HUMAN PROSTATE CANCER CELLS

Jiun-Long Yang[1], Chao-Lin Kuo[2] and Jing-Gung Chung[3]

[1]Graduate Institute of Chinese Pharmaceutical Science, [2]School of Chinese Medicine Resources, [3]Department of Biological Science and Technology, China Medical University, Taichung, Taiwan, ROC

Prostate cancer is the one of the major causes of death by cancer in older man worldwide. *Euphorbia formosana* radix (EF_R) has long been used as an anti-snake venom in traditional Taiwanese medicine. However, there is no available information to address the effects of EF_R on human prostate cancer cells. In this study, we investigated the cytotoxicity of EF_{REC} in human prostate cancer DU 145 cells. The results showed that EF_{REC} decreased the percentage of viable DU 145 cells in dose- and time-dependent manners. The IC_{50} of EF_R is 50 μg/ml and this concentration was used for further study. Flow cytometry was used for examining cell cycle arrest and the proportion of sub-G1 DU 145 cells and results showed that EF_{REC} induced significant S-phase arrest and sub-G1 groups appear. Comet assay demonstrated that EF_{REC} induced significant DNA damage in DU 145 cells and DNA gel electrophoresis indicated that EF_{REC} induced apoptosis due to the present of DNA fragmentation. EF_{REC} Induced the productions of cytosolic reactive oxygen species (ROS) and calcium ions but reduced the changes in mitochondria membrane potential ($\Delta\Psi m$) in examined cells. Results from Western blotting indicated that EF_{REC} reduced pro-caspase-3, pro-caspase-9, pro-caspase-8 and Bcl-2, but increased Bax, cytochrome-*c*, Endo-G, catalase, glutathion transferase, SOD-2, caspase-3, caspase-8 and casepase-9 proteins. In conclusion, EF_{REC} induced apoptosis of DU 145 cells through mitochondria- and caspase-3-dependent pathways.

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HUMAN CELLS COMMONLY ACQUIRE DNA DAMAGE DURING MITOTIC ARREST

W. Brian Dalton, Mandayam O. Nandan and Vincent W. Yang

Division of Digestive Diseases, Department of Medicine, Emory University School of Medicine, 615 Michael Street, Atlanta, Georgia 30322, USA

The mitotic checkpoint is a mechanism which arrests the progression to anaphase until all chromosomes have achieved proper attachment to mitotic spindles. In cancer cells, satisfaction of this checkpoint is frequently delayed or prevented by various defects, some of which have been causally implicated in tumorigenesis. At the same time, deliberate induction of mitotic arrest has proven clinically useful, as antimitotic drugs which interfere with proper chromosome-spindle interactions are effective anticancer agents. However, how mitotic arrest contributes to tumorigenesis or antimitotic drug toxicity is not well defined. Here we report that mitotic chromosomes can acquire DNA breaks during both pharmacological and genetic induction of mitotic arrest in human cancer cells. These breaks activate a DNA damage response, occur independently of cell death, and subsequently manifest as karyotype alterations. Such breaks can also occur spontaneously, particularly in cancer cells containing mitotic spindle abnormalities. Moreover, we observed evidence of breakage in primary human cells. Our findings thus describe a novel source of DNA damage in human cells. They also suggest that mitotic arrest may promote tumorigenesis and antimitotic toxicity by provoking DNA damage.

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HIPPOCRATES OF KOS, THE FATHER OF CLINICAL MEDICINE AND ASCLEPIADES OF BITHYNIA, THE FATHER OF MOLECULAR MEDICINE

Christos Yapijakis

University of Athens Medical School, Athens, Greece

Hippocrates of Kos (460-377 BC) is universally recognized as the father of modern medicine, which is based on observation of clinical signs and rational conclusions. Before him therapeutic attempts were based on religious or magical beliefs. He belonged to a family of physicians who claimed their ancestry from Asclepius, the god of medicine. Hippocrates worked mainly in the Aegean island of Kos and the nearby Minor Asia coast, but he also traveled extensively visiting Athens, Thessaly and Thrace. His contribution to medical practice (collectively found in the *Hippocratic Corpus*) is characterized by ethical rules of conduct (*Hippocratic Oath*), close observation of clinical symptoms, an open mind for any ideas, and a willingness to explain the cause of diseases. Hippocrates and his followers, for about two and a half millennia, based medicine on the notion that nature was made of four elements (established by the philosopher Empedocles), namely water, earth, wind and fire. In an analogous way, the body consisted of four fluids or "humors" (black bile, yellow bile, phlegm and blood) and four elemental conditions (cold, hot, dry and moist). The state of health according to Hippocrates existed when these humors and qualities were in balance. The physician had to make a diagnosis and then facilitate the healing work of "benevolent Nature" by use of bleeding or purgatives. Despite some wrong assumptions, the contribution of Hippocrates to clinical medicine is immense. The clinical and ethical basics of medical practice, as well as most clinical terms used even today have their origins in Hippocrates.

Asclepiades of Bithynia (124-40 BCE) was the first physician who spoke about what is known today as molecular medicine. Asclepiades was the first famous physician who established Hellenic Medicine in Rome. He was born in Prousa, Bithynia in the northwest region of Minor Asia, he was educated at the Epicurean School in Athens and at the age of 33 years he moved to Rome, where he first taught philosophy and later on practiced medicine. In contrast to the Hippocratic dogma of four elements and humors, he adhered to atomic theory, change and evolution, and did not accept the theory of a "benevolent Nature". He suggested that the human body is composed of void spaces (*poroi*), as well as molecules (*meri* or *corpuscula*) made of atoms (*anarmoi ongoi*). According to Asclepiades, diseases are caused by alteration of form or position of a patient's molecules. In order to restore health, he favored mild therapeutic methods such as healthy diet, exposure to light, hydrotherapy, massage, physical exercise, and above all the friendly support of patients. Asclepiades was the first physician who made the highly important division of diseases into acute and chronic ones and was the first to perform an elective non-emergency tracheotomy. He was a pioneer in treating women (previously thought to be inferior beings) and in the humane treatment of patients with mental disorders, using labor and music therapy. His humane and naturalistic approach, as well as his medical skills gave him a great reputation. His influence lasted for six centuries through the Methodic Medical School, which was established by his students. Some ideas of Asclepiades have been rediscovered in the past century and represent the molecular basis of medicine.

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INHERITED GENE POLYMORPHISMS OF FACTORS RELATED TO ANGIOGENESIS, INFLAMMATION AND THROMBOSIS THAT INFLUENCE RISK FOR ORAL CANCER

C. Yapijakis[1,2], D. Avgoustidis[1], Z. Serefoglou[1], A. Vylliotis[1], S. Spyridonidou[1], E. Critselis[1], E. Nkenke[2], FW. Neukam[2], E. Patsouris[3] and E. Vairaktaris[1]

Departments of [1]Oral and Maxillofacial Surgery, [2]Neurology and [3]Pathology, University of Athens Medical School, Athens, Greece;
[4]Department of Oral and Maxillofacial Surgery, University of Erlangen, Nürnberg, Germany

Introduction: Recent genetic association studies performed by our group and others have provided evidence that inherited functional DNA polymorphisms in genes of factors related to angiogenesis, inflammation and thrombosis are associated with increased risk for oral squamous cell carcinoma (OSCC). The present study examined the possible combinatory effect of 31 such gene polymorphisms in predicting the occurrence of OSCC in Europeans using multivariate logistic regression analysis. *Materials and Methods:* A total of 330 individuals of Greek and German origin were studied, consisting of 162 OSCC cases and 168 healthy controls of comparable age, gender, and ethnicity. DNA was isolated from blood samples of studied individuals. Functional DNA polymorphisms in 31 genes that encode cytokines and their receptors, matrix metalloproteinases and their inhibitors, platelet glycoproteins and coagulation factors were investigated. They included *IL1b (+3953C/T), IL-4 (-590C/T), IL-6 (-174G/C), IL-8 (-251A/T), IL-10 (-1082A/G), IL–18 (-607C/A), TNF-α (-308G/A), TNF-β (+252G/A), VEGF (+936C/T), Leptin (-2548G/A), Leptin Receptor (223Gln/Arg), MMP-1 (-16071G/2G), MMP-3 (-11715A/6A), MMP-7 (-181A/G), MMP-9 (-1562C/T), MMP-13 (-77A/G), TIMP-2 (-418C/G), GPIα (807C/T), GPIbα (VNTR), PAI-1 (4G/5G), AGT (325Met/Thr), ACE (intron 16D/I), TAFI (+325C/T), Thrombomodulin (-33G/A), Protein Z (-13G/A), SDF1 (801G/A), Factor II (20210G/A), Factor V*

(1691G/A), Factor XII (46C/T), Factor XIII (34Val/Leu), and *MTHFR (677C/T)*. A series of regression models (adjusted for age and gender) was constructed in order to assess the contribution of homozygous or heterozygous variant polymorphic genotypes upon overall, early and advanced stages of OSCC development. *Results:* In almost all multivariate logistic regression models, the contribution of TNF-α and IL-6 polymorphisms was consistent and robust. Furthermore, when the mode of inheritance of each variant allele was taken into account in a model, five polymorphisms emerged as primary predictors for all OSCC stages: TIMP-2 (OR=26.33; 95%CI=12.39–55.95), TNF-α (OR=15.27; 95%CI=7.30–31.96), IL-6 (OR=8.33; 95%CI=3.95–17.58), IL-8 (OR=3.54; 95%CI=1.69–7.43) and IL-10 (OR=2.65; 95%CI=1.28–5.46). *Conclusion:* The present regression analysis revealed a highly significant contribution of 5 out of 31 studied factors in the occurrence of OSCC. Based on these findings and previous reports, possible interactions of the implicated factors leading to OSCC development, as well as an algorithm of risk estimation will be discussed.

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EFFECT OF *CORDYCEPS SINENSIS* ON SPONTANEOUS METASTATIC MODEL MICE

Noriko Yoshikawa, Erika Kubo, Satomi Kagota, Kazumasa Shinozuka, Masaru Kunitomo and Kazuki Nakamura

Department of Pharmacology, School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya, Hyogo 663-8179, Japan

Cordyceps sinensis has been used as a herbal tonic in traditional Chinese medicine. We previously reported that the water extract of the fruiting bodies of cultured *Cordyceps sinensis* (WECS), when administered to a hematogenic lung metastatic mouse model concomitantly with methotrexate (MTX), a folic acid antagonist, indicated a combined anti-metastatic effect. In this study, we investigated the effect of WECS on a spontaneous metastatic mouse model, and also examined the combined effect of hydroxyurea, a ribonucleotide reductase inhibitor, with WECS. Highly metastatic B16-BL6 mouse melanoma cells (1×10^6) were injected subcutaneously into the footpad of the right hind leg in syngeneic C57BL/6Cr mice. Two weeks after inoculation, the mice were anesthetized with diethyl ether and the massively enlarged primary tumor was amputated. WECS (3 and 10 mg/kg/day) and hydroxyurea (500 mg/kg/day) were intraperitoneally administered to the mice for 7 days after inoculation and for another 7 days after amputation. To evaluate the anti-metastatic effect of WECS, we measured the survival of mice. As a result, mice that had been administered WECS 10 mg/kg showed significantly prolonged survival compared with that in control mice. Furthermore, hydroxyurea reinforced the anti-metastatic effect of WECS. In conclusion, WECS may be useful for the treatment of metastatic tumors, and a promising adjuvant with conventional anti-tumor agents such as hydroxyurea.

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THE ROLES OF REACTIVE OXYGEN SPECIES AND CALCIUM IN CAPSAICIN-INDUCED APOPTOSIS IN MDA-MB-231 HUMAN BREAST CANCER CELLS THROUGH MITOCHONDRIA-DEPENDENT PATHWAY

Chun-Shu Yu[1], Yu-Ping Lin[2], Hsu-Feng Lu[3] and Jing-Gung Chung[4]

[1]School of Pharmacology, Departments of [2]Nutrition and [4]Biological Science and Technology, China Medical University, Taichung, Taiwan;
[3]Department of Clinical Pathology, Cheng Hsin Rehabilitation Medical Center, Taipei, Taiwan, ROC

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), the main pungent ingredient of red pepper, has been reported to possess biological activities such as anticarcinogenic and anticancer activities. In the present study, the effects of capsaicin on human breast cancer MDA-MB-231 cells and the signaling pathways involved in apoptosis it induces were investigated. Treatment of MDA-MB-231 cells with capsaicin inhibited cell growth and induced apoptosis through reactive oxygen species (ROS) and Ca^{2+} generation, down-regulation of Bcl-2 and activation of caspase-3. The increases in both Ca^{2+} and ROS production were abolished by BAPTA (Ca^{2+} chelator) and *N*-acetylcysteine (ROS scavenger) and pretreatment with both compounds also prevented capsaicin-induced cell death. Capsaicin-promoted activation of ERK was prevented with all the inhibitors tested. Interestingly, capsaicin induced morphological alterations and reduced the percentage of viable cells. Moreover, capsaicin increased the protein levels of Bax, cytochrome *c* and active-caspase-3 but reduced the levels of Bcl-2 and Bcl-xl. We conclude that capsaicin induces apoptosis in MDA-MB-231 cells *via* ROS and Ca^{2+} generation and caspase-3 activation.

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NOTI MICROARRAYS (NMA) FOR EPIGENETIC PROFILING OF CANCER CELLS

V. Kashuba, T. Pavlova, K. Haraldson, I. Skrypkina, S.P. Yenamandra, V. Senchenko, V. Zabarovska, E. Braga, T. Eschenko, E. Grigorieva, M.I. Lerman, G. Klein and E. Zabarovsky

MTC, Karolinska Institute, Stockholm, Sweden

We have developed restriction site tagged microarrays (RSTM) and used NotI microarrays (NMA) for pilot

experiments. Creation of microarrays on the genomic level would be very important as it may give information unavailable on mRNA/cDNA level (*e.g.* methylation, histone acetylation, hemizygous deletions, epigenetic factors). NMA are the only existing microarrays giving the opportunity to detect simultaneously and differentially copy number and methylation changes. Thus they allow to check cancer cells for genetic and epigenetic abnormalities. These NMA can be used for individuals, normal/tumor pairs, different cell types etc. At present, we analyzed over 400 samples representing various cancers: breast, kidney, cervical, colon, ovarian, lung, prostate, nasopharyngeal carcinoma and leukemia. In the study 190 genes from human chromosome 3 were analyzed. For all studied cancers we found genes specifically methylated in malignant cells. Many genes were found to be methylated in a very high fraction of cancer samples (more than 80%). These genes can be divided in two classes: cancer specific and common for several types of cancer. Some examples of genes involved in several cancers: MINT24 (AF135524), BHLB2, LOC285205, NBEAL2 (KIAA0540), FLJ44898 (AK126846), GATA-2, RARbeta1, RBSP3 (CTDSPL), VHL. Many methylated genes were unknown previously to be involved in the development of epithelial cancers. Methylation of more than 15 genes was confirmed by methyl-specific PCR and bisulfite sequencing. Methylation status of the genes correlated with expression (more than 15 genes were tested). For example, expression of RBSP3 was strongly suppressed (more than 100-fold) in cervical samples and degree of the expression decrease was tumor progression dependent. Four genes were tested functionally and demonstrated growth inhibiting activity proving that NMA are efficient instrument to discover cancer causing genes and especially tumor suppressor genes. We found that several tumor suppressor genes in AP20 and LUCA 3p21.3 regions were co-regulated in tumors. For example, real-time PCR was used to measure mRNA level of RBSP3, NPRL2/G21, RASSF1A, ITGA9, HYAL1 and HYAL2 in basic types of NSCLC – squamous cell lung cancer (SCC, 41 samples) and lung adenocarcinoma (ADC, 18 tumors). Significant (from 2 to 100 times) and frequent (from 44 to 100%) mRNA level decrease was shown in the tumors. Down-regulation of RASSF1A and ITGA9 was associated significantly with ADC progression. Simultaneous decrease of all six genes' mRNA level was found in the same tumor samples and was not depended on their localization in 3p21.3 and functions of the proteins. These results supported the hypothesis on simultaneous inactivation of cluster cancer-causing genes in AP20 and LUCA regions during the development and progression of lung cancer and other epithelial tumors. The data could be important for development of specific biomarker sets for early cancer diagnosis and new therapeutic approaches/strategies for cancer treatment. Genes specifically demethylated in tumors were also found. They could represent oncogene-like genes.

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COMBINATION OF SYNTHETIC ORGANOSILICON COMPOUNDS (SILA-409 AND SILA 421) WITH CYTOSTATIC DRUGS IN XENOGRAFT MODELS OF HUMAN PANCREATIC CANCER

Attila Zalatnai[1] and József Molnár[2]

[1]First Department of Pathology and Experimental Cancer Research, Semmelweis University, Faculty of Medicine, 26 Üllői Rd., Budapest, Hungary, H-1085;
[2]University of Szeged, Department of Medical Microbiology and Immunobiology, Szeged, Hungary

Introduction: Pancreatic cancer is characterized by an aggressive behavior, poor prognosis and the main obstacle is its chemoresistance. In more than 70% of these tumors the P-glycoprotein encoded by the Multidrug-resistance (MDR)- gene is overexpressed. Several mdr-revertant molecules have been synthesized, among them the patented organosilicon compounds (SILA-409, SILA-421) showed promising *in vitro* and *in vivo* activities. We have investigated the combination of these compounds with various cytostatic drugs to achieve a better tumor-inhibiting effect. *Materials and Methods:* Human pancreatic cancer xenografts (PZX-40) subcutaneously growing in immunosuppressed mice have been continuously treated with intraperitoneal administration of SILA-409 and SILA-421, together with gemcitabine, vincristine, irinotecan or paclitaxel for a month. Tumor volumes were weekly calculated, the expression of P-170 and Ki-67 were assessed by immunohistochemistry. *Results:* These organosilicon compounds are not toxic in mice even at high doses (10 mg/kg) and they are well tolerable drugs. Although gemcitabine is not a P-gp substrate, combination with these organosilicons resulted in a tumor growth delay, a volume reduction or volume stabilization. Growth delay was also found in SILA + vincristine, SILA + paclitaxel groups, but not after SILA + irinotecan treatment. Irinotecan by itself has abolished the P-gp expression, but the other combination treatments all have led to significantly diminished P-170 immunopositivity. The Ki-67 proliferative index was reduced in all treated tumors. *Conclusion:* SILA-409 and SILA-421 synthetic organosilicon compounds showing an MDR-revertant activity *in vitro*, were also effective in *in vivo* experimental systems. They can be administered safely, with no side-effects. Decreased P-gp expression was also demonstrated in the tumors. These drugs can be effectively combined with cytostatics. The organosilicon compounds are promising molecules in the oncology research.

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UNDERLYING MOLECULAR ALTERATIONS IN PROGRESSION OF NSCLC

I. Zborovskaya, K.K. Lactionov, B. Polotsky, D. Yudin, I. Favorskaya, E. Musatkina, M. Kochetkova, O. Kovaljova, V. Mochalnikova, K. Arhipova, E. Stepanova, B. Ahmetov and A. Telezhkina

N.N.Blokhin' Cancer Research Center of RAMS, Moscow, Russia

Molecular changes, during different steps of tumor progression were compared in group of tumors with different morphology and level of cell differentiation. The significance of individual gene alterations and certain set of molecular markers in clinical trials were evaluated in various stages of non-small cell lung cancer in patients radically operated in Blokhin's RCRC of RAMS in 1985-2006 (RAS gene polymorphism, deletion in loci 1p32-36, 7q31 and 11p15, expression of MMPs, CAV-1, BIRC5, CDH1, CTNNB1, uPA/uPAR, RALA, DAPk1, BCL-2, BAX, p53, VEGF/VEGFR *etc*.). These results point to a subgroup of tumors with complex molecular abnormalities that correlate with worst prognosis. The study focuses on the prognosis of 120 patients with different metastatic status. The panel of molecular markers consisted of deletion in loci 1p32-36, expressions of mutant p53, BCL-2, BAX, VEGF. The simple statistical and regressive-factorial analysis of molecular markers shown in NSCLC indicated that expression of mutant p53 and VEGFa, deletion in 1p32.1 and 1p36.3 and reduction of BAX expression can be reliable adverse prognostic factors for NSCLC. On the basis of the calculated values of weight factors and clinical, morphological and molecular-biological markers, it is possible to differentiate between patients with poor (less two years) and favorable prognosis (more than five years).

The results allow the individual prognosis of survival rate for each patient with NSCLC. The potential clinical utility of these findings and the possible direction for further study are discussed.

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CONTROVERSIES IN THE MANAGEMENT OF OVARIAN CANCER PRO AND CONS OF INTRAPERITONEAL (IP) CHEMOTHERAPY

Alain G. Zeimet

Department of Obstetrics and Gynecology, Innsbruck Medical University, Austria

Ovarian cancer generally remains confined to the abdominal cavity throughout its course. Three randomized trials have shown superiority of intraperitoneal (IP) over conventional intravenous (IV) chemotherapy in optimally debulked patients(residual disease ≤1 cm). Thus, in the last two years the route of administration of the cytotoxic agents became a subject of major debate in the treatment of ovarian cancer. Based on the outcome of the International Consensus Meeting on IP Chemotherapy in Ovarian Cancer held in Innsbruck in February 2006, in our institution, IP chemotherapy is routinely applied in appropriate patients and we even extended the indications for IP chemotherapy from FIGO stage III to earlier stages. The two-years experience of the Austrian-wide AGO observational study will be reported. A steeply increasing learning curve in adequate catheter implantation, in the management of complications with IP administration and treatment-related toxicities were noticed. The most mentioned side-effects were long lasting neurotoxicity, abdominal pain, fatigue, gastro-intestinal, metabolic toxicities and infrequently catheter-related complications. Apart from side-effects related to the catheter, most toxicities were identified to mirror the toxicity profile of high-dose cisplatin (≥100 mg/m^2) and were independent from the route of drug administration. Therefore the classic cisplatin/paclitaxel IP-regimen used in the GOG-172 trial was changed to an alternative regimen comprising carboplatin AUC 5 (d.1) and weekly paclitaxel 60 mg/m^2 (d.1,8,15) In this therapeutic approach all drugs were administered *via* intraperitoneal route. This treatment was by far better tolerated and quality of life, during and after therapy, was significantly less compromised. However, the toxicity profile of this novel approach was different from that observed when the included drugs were given intravenously. The most relevant and limiting side-effect of this IP-regimen was myelotoxicity with a high rate of thrombocytopenia leading frequently to a delay in drug administration. Furthermore, new data on cost effectiveness comparing both IP and IV chemotherapy in advanced ovarian cancer will be discussed on the basis of comparable large randomized IV and IP GOG-trials. *Conclusion:* In cases, where optimal cytoreduction with residual disease ≤1 cm was achieved during primary surgery and disease was confined to the peritoneal cavity, IP-chemotherapy should seriously be taken into consideration even at the expense of significantly increased, but manageable toxicity. A more favorable therapeutic index should be expected in IP regimens, when cisplatin is substituted by the better tolerable carboplatin.

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ANTICANCER ACTIVITIES OF WILD AND CULTIVATED EDIBLE DANISH MUSHROOMS

Lin Zhai[1], Ming Chen[1], Henning Knudsen[2], Henrik Hoff-Hansen[3] and Arsalan Kharazmi[1]

[1]Department of Clinical Microbiology, 7602, University Hospital of Copenhagen (Rigshospitalet), Tagensvej 20, Copenhagen, DK-2200 N;

[2]Botanical Museum, Gothersgade 130, Copenhagen, DK-1123 K;
[3]Rosbaeksvej 4, DK-2100 Copenhagen Ø, Denmark

Coprinus comatus, Meripilus giganteus, Flammulina velutipes, Grifola frondosa, and other 17 wild edible Danish mushrooms were collected from North Zealand, Denmark. *Agaricus bisporus, Pleurotus eryngii, Pleurotus ostreatus,* and other three cultivated mushrooms were purchased from Danish supermarkets. Fresh mushrooms were dried in an atmosphere of 37˚C ventilation. Water extractions of these mushrooms were made and were sterile filtered through a 0.22-μm-pore-size Millipore filter.

The *in vitro* anticancer activity of the water extractions of these mushrooms have been tested against four different cancer cell lines, K562, EL_4 (both are leukemic cell lines), MCF_7 (human breast cancer cell line), and PC_3 (human prostate cancer cell line). A standard assay (sulforhodamine B staining method) for anticancer-drug screening recommended by National Cancer Institute has been employed for the study.

The results show that some mushrooms exhibit potent inhibitory activity on the *in vitro* growth of the four different cancer cell lines. Among the tested wild edible Danish mushrooms, *C. comatus, G. frondosa*, and *M. giganteus,* show potent *in vitro* anticancer activity against all four different cancer cell lines. At the concentration of 0.01 g/ml, *G. frondosa* and *F. velutipes* inhibited the *in vitro* growth of EL-4 cell line by 92% and 70%, respectively. *A. bisporus, P. eryngii, P. ostreatus* and other three cultivated edible Danish mushrooms exhibit moderate inhibitory effect on the *in vitro* growth of K562 and EL_4, and slight effect on PC_3. However, they have no effect on the *in vitro* growth of MCF_7. Preliminary mechanism of action study indicates that the *in vitro* anticancer activity of these mushrooms is probably *via* apoptosis.

In many parts of the world, wild mushrooms are regularly collected and used directly as a main source of food or added to soups, stews and teas. Our data indicate that some wild edible Danish mushrooms have potent anticancer activity *in vitro* and there is a great potential to find new anticancer agents from these mushrooms. Recommendation of these edible mushrooms to cancer patients as functional foods should be discussed.

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INDUCTION OF MEGAKARYOCYTIC DIFFERENTIATION AND GROWTH INHIBITION OF K562 ERYTHROLEUKEMIA CELLS BY OVER-EXPRESSION OF CYP2E1

Weiqiang Zhao, Yan Tang, Maureen E. Baird, Gerard Lozanski and Frederick K. Racke

Department of Pathology, Ohio State University, Columbus, OH 43210, USA

Cytochrome P450 2E1 (CYP2E1), found predominantly in hepatic cells involved in xenobiotic metabolism, has been occasionally found to be up-regulated in other cells, including certain types of leukemia. In the current study, the role of CYP2E1 in leukemic K562 cells was investigated. Over-expression of CYP2E1 reduced proliferation compared to empty vector control cells. Cell cycle analysis indicated CYP2E1 cells produced maximum G_2/M arrest of 33% and 28% at 24 hr and 48 hr, respectively, in comparison with control cells (10% and 19%, 24 and 48 hr, respectively). Growth arrest was accompanied by induction of megakaryocytic differentiation as evidenced by cell enlargement, polyploidization, and CD41 expression. Induction of megakaryocytic differentiation of K562 cells by phorbol myristate acetate (PMA) was accompanied by increased expression of CYP2E1 at both the protein and mRNA levels. Expression of BCR-ABL mRNA was not affected by overexpression of CYP2E1; however, the pBCR-ABL and pSTAT5 were suppressed in CYP2E1 in overexpressors compared to control cells. When CYP2E1 K562 was treated with a dual kinase inhibitor, NS187 at 1 μM, a synergistic inhibitory effect on cell growth was observed at all time points checked from 24 to 72 hr. The mechanisms of effect of ectopic expression of CYP2E1 in K562 cells are not understood yet; however, recent studies have shown that production of reactive oxygen species (ROS) in normal hematopoietic progenitors promotes megakaryocytic differentiation. Therefore, we postulate that over-expression of CYP2E1 might increase ROS production and this will be tested in the future studies. In summary, over-expression of CYP2E1 in K562 erythroleukemia cells resulted in a cell growth arrest due to a G_2/M phase arrest, megakaryocytic differentiation, and a synergistic cell growth inhibition with pBCR-ABL inhibitor. The results from this study might have clinical implications. For instance, drugs that inhibit CYP2E1 activity might inhibit megakaryocytic growth and be applied in the treatment of myeloproliferative disorders with abnormal megakaryocytic proliferation; in contrast, strategies to increase CYP2E1 activity might be applied to promote thrombopoeisis in cases of the acquired thrombocytopenia. Finally, strategies to increase CYP2E1 activity may act synergistically with bcr-abl kinase inhibitors as novel therapy for Ph+ leukemia.

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BLOOD TEST FOR CUTANEOUS MALIGNANT MELANOMA

M. Ziman, S. Medic, R. Slattery and R. Pearce

School of Exercise, Biomedical and Health Science, Edith Cowan University, Joondalup, Perth, 6027, Australia

Finding sensitive markers to detect dissemination of metastatic melanoma cells is an important research focus as

metastasis may be undetected clinically and recurrence can occur many years after surgery.

Here we have used RT-PCR and real-time RT-PCR to assess the presence of several molecular markers of circulating melanoma cells in peripheral blood from 200 melanoma patients and 50 healthy volunteers. The frequency and level of expression of markers was correlated to Breslow tumour thickness and tumour progression, and results were statistically analysed.

Results show that markers of circulating melanoma cells were detected in blood of 79% of melanoma patients and in only 11% of healthy volunteers. Several markers showed a higher detection rate overall, regardless of tumour thickness, although levels were highest in those patients with thicker tumours. Notably, migrating melanoma cells were found in peripheral blood of patients with early-stage tumours and in patients from whom tumours were removed several years previously. Further research is progressing to increase the sensitivity of detection and characterise the gene expression profile of migrating metastatic melanoma cells.

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HYPERTHERMIC ISOLATED LIMB PERFUSION (HILP) WITH MELPHALAN PLUS TNF

O. Zoras, C. Ioannou, S. Koukouraki, K. Perisynakis, A. Theodosakis, Th. Moschovos and G. Chalkiadakis

Surgical Department, University of Crete Medical School, Herakleion, Crete, Greece

Introduction: After completion of a trial period in which leakage control and surgical skills were assessed by Experts in the Surgical Department, University of Crete, our team was accreditated the use of TNF (Beromun) in local regional treatment for inoperable or recurrent sarcomas and melanomas of the extremities. *Purpose:* To depict our experience, as well as our technique with the use of Melphalan and TNF in HILP in inoperable or recurrent sarcomas and melanomas of the extremities as performed in the Surgical Department, University of Crete between 2006 and today. *Patients and Methods:* A total of eight cases were treated. Three sarcomas of which 1 leiomysarcoma located just above the left external malleolus, 1 leiomysarcoma in the right tibia and the last, a liposarcoma, in the right femorus. All of the previous cases were initially treated elsewhere and were referred to us after recurrence. Furthermore, five intransit melanomas of the lower limb were treated. Chemotherapy dosages for Melphalan were calculated according to common protocol (10 mg/liter of limb volume) and TNF was 2 mg in total dose. All patients were recovered in the ICU for 24 hours in accordance to protocol. *Results:* Although statistical comparison is not applicable, we observed a notable shrinkage of the tumor and a very good partial response of the sarcomas and complete response of the melanomas. No systemic toxicity was observed. Limb toxicity as measured by the Wieberdink scale was 0 in six cases and 2 in two cases. Functional disability was minimal in all cases. *Conclusion:* Our experience verifies that the use of Melphalan and TNF in HILP is safe when guidelines and safety rules are followed.

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GENE AND STEM CELL CANCER THERAPY USING TRAIL

Ralf Zwacka[1,2], Chirlei Bueneker[1], Laura Deedigan[1] and Andrea Mohr[1]

[1]NCBES, Molecular Therapeutics Group, National University of Ireland, Galway, and [2]Regenerative Medicine Institute (REMEDI), University Road, Galway, Ireland

The tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is a potent inducer of apoptosis in cancer cells. We have tried several ways of delivering TRAIL to tumours including adeno-associated viral (AAV) vectors. Recently we have used mesenchymal stem cells (MSCs) to deliver TRAIL to sites of malignant growth. The prerequisite for the use of MSCs is that they can withstand the apoptosis-inducing activity of TRAIL. Indeed, we found that MSCs are completely resistant to the effects of TRAIL-induced apoptosis. Analyses of TRAIL receptor expression revealed that MSCs do not have any detectable levels of the four TRAIL-receptors on their surface. The forced expression of TRAIL-R1 in MSC using an adenoviral vector rendered them sensitive to TRAIL, demonstrating that lack of TRAIL-receptor expression is the cause for the observed unresponsiveness. Co-treatment of MSCs with TRAIL and IFN-γ or 5-FU showed no increase in the apoptotic response, pointing to the safety of potential future TRAIL-based cancer therapies even in combination with other anti-tumour agents. We then transduced MSCs with an adenoviral vector expressing TRAIL (Ad.TR) and showed the apoptosis-inducing activity of these TRAIL-carrying MSCs on A549 lung carcinoma cells, which are normally resistant to the effects of recombinant TRAIL protein. We observed the same effect in various other types of cancer cells. Apoptosis was also induced in A549 cells by Ad.TR-transduced MSCs in the presence of physiological concentrations of white blood cells, erythrocytes and sera from human donors, factors that often inhibit conventional viral vector approaches. Moreover, we demonstrated tumour growth reduction with TRAIL-loaded MSCs in an A549 xenograft mouse model. However, despite these encouraging results several hurdles exist for successful TRAIL-based therapies. These include the

presence of several anti-apoptotic NF-κB target genes such as *XIAP, Bcl-x_L, c-Flip* and *MnSOD* in cancer cells. We found varying contributions of these factors to apoptosis resistance and we are currently trying to identify which of these factors are good targets for inhibitory approaches that could be combined with our gene-cell therapeutic tumour targeting strategies.

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AIR POLLUTION AND CANCER

Michael Theophanides[1], Jane Anastassopoulou[2] and Theo Theophanides[2]

[1]CAE, Saint Laurent, Montreal, Quebec, Canada H42 4x4; [2]National Technical University of Athens, Chemical Engineering Department, Radiation Chemistry and Biospectroscopy, Zogragou Campus, 15780 Zografou, Athens, Greece

Air Quality Studies are becoming an essential element of operations, since the chemical pollutants present serious health concerns. The existence of human, animal and plant life on earth is dependent upon keeping the atmosphere in a reasonable state of purity. This paper presents a comparative study of mortality statistics attributed to cancer, cardiac and pulmonary diseases in the last 30 years in Kavala, other cities of Greece, and at the National level. The objective of this work is to demonstrate the above-average trends in mortality due to cancer, cardiac and pulmonary diseases in areas of high industrial pollution attributed to air pollution, water pollution and food contamination. Statistical analysis of the data indicates that the prefecture of Kavala, Greece has a higher rate of mortality (per 1000) by approximately 20% due to cancer, cardiac and pulmonary diseases compared to the National average. There is also a trend indicating a growing disparity of death rates between the National average and polluted areas. Other trends will be analysed that show the increase in the percentage of cancer-related mortalities compared to total mortalities in polluted areas. For example, in 1980, Kavala had a lower percentage (30%) of mortalities attributed to cancer for ages less than 65 years old compared to the National average (36%). However, in 1998, Kavala had an equal amount of percentage of mortalities (36%) attributed to cancer compared to the National average (28%) indicating that the significant decrease during 1980-1998 in cancer-related mortalities for younger age groups represented in the National average are not exhibited for the Kavala area. For the higher age group of 65 to 74 years old, the results show that while the National average has remained steady at 32%, the Kavala average has *increased* during 1980 to 1998 from 36% to 40%.

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THE IMPORTANCE OF BIOMARKERS IN PROSTATE CANCER DIAGNOSTICS (A PILOT STUDY)

L. Holubec, J. Vrzalova, J. Klecka, M. Pesta, O. Topolcan, V. Eret and M. Hora

University teaching hospital, Plzen and Charles University of Plzen, E.Benese 13, 305 99, Plzen, Czech Republic

Background: Early diagnosis of prostate cancer (PCa) in organ confined stage with following radical treatment is the only potential currative approach in PCa. PSA is a leading marker in diagnosis, follow up, and therapy control. The aim of the present pilot study was to evaluate diagnostic potential of selected biomarkers for Pca diagnostics. *Materials and Methods:* We examined altogether 66 patients. 27 patients with benign prostatic hyperplasia (BPH), 9 patients with prostatic intraepithelial neoplasia (PIN) and 30 patients with prostate cancer (Pca). By means of immunoanalytical methods and methods of multiplex analysis we assessed the following biomarkers: PSA, TK, Chromogranin A, ICAM-1, VCAM-1, MMP-9, VEGF, IL-6, IGFBP3, IGF1. *Results:* Patients with BPH and patients with PCa exhibited statistically significant differences in the levels of PSA (0.0001), IGFBP3 (0.0027) and IGF1 (0.0148). We found also statistically significant differences between patients with BPH and PIN in the levels of PSA (0.0271), VEGF (0.0235) and TK (0.0426). IGFBP3 was also significantly higher in patients with PCa in comparison to patients with PIN. The levels of PSA, TK and Chromogranin A correlated with stage of disease, and levels of MMP-9 correlated with Gleason score of prostate cancer. *Conclusion:* The results of the present pilot study point out that PSA is a leading biomarker for the diagnosis of prostate cancer. But also other biomarkers can improve diagnostic accuracy and prognosis. This work was supported by grant IGA NR/8918-3 and by the Research project VZ MSM 0021620819.

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MECHANISMS OF CHEMOTHERAPY RESISTANCE IN HEAD AND NECK CANCER

Robert Mandic

Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Giessen and Marburg, Campus Marburg, Germany

Head and neck squamous cell carcinomas (HNSCCs) account for over 90% of all malignancies in the upper aero-digestive tract. Despite multimodal treatment options that include surgery, radio- and chemotherapy overall survival of HNSCC patients did not improve to a significant extent

within the past 30 years. The major cause for lack of improvement lies in the development of tumor cell resistance to treatment by radio- and chemotherapy. Particularly after exhausting radiotherapy, chemotherapy often remains the only treatment option. Cisplatin is the major compound used in HNSCC chemotherapy treatment, therefore resistance to platinum-based compounds is of central interest. In this short survey we discuss the problem of chemotherapy resistance in head and neck cancer treatment. The underlying mechanisms conferring chemotherapy resistance to HNSCC cells such as overexpression of antiapototic proteins, p53 mutations and up-regulation of efflux pumps will be pointed out. Lastly the role of cancer stem cells (CSCs) in the development of tumor cell resistance will be discussed. New targeting strategies focusing on CSCs are an exciting new perspective for future anticancer research.

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AN OPERATIVE GAMMA CAMERA DEDICATED TO THE OPTIMIZATION OF SENTINEL LYMPH NODE PROCEDURE IN BREAST CANCER SURGERY

[1]Z. Francis, [1]V. Bekaert, [1]S. Salvador, [1]D. Brasse, [1]D. Huss, [2]C. Mathelin

[1]Institut pluridisciplinaire Hubert Curien, 23 avenue du Loess, 67037 Strasbourg;
[2]Unité de sénologie, CHRU, Hôpitaux Universitaires de Strasbourg, 1 place de l'hôpital, 67091 Strasbourg Cedex, France

A close collaboration between the "Hôpitaux Universitaires de Strasbourg" (Strasbourg, France) and the "Institut Pluridisciplinaire Hubert Curien" (Strasbourg, France) led to the development of a small gamma camera covering a field of view of 100 °— 100 mm2, dedicated for breast cancer surgery. This on-demand device is dedicated to per-operative imaging aiming to identify and localise the Sentinel Lymph Nodes (SLN). Nowadays, surgeons try to use the SLN procedure avoiding the classical axillary lymph node dissection which causes several side effects notably lymphoedema after surgery. The procedure (used in 70% of breast cancer cases) consists on localising the SLN as precisely as possible and taking them off making sure no residual SLN remains in the axillary area. Our first experiment was a small field gamma camera (50 °— 50 mm2) built according to the dimensions of the H8500 Hamamatsu's multianode photomultiplicator. Its small size makes it flexible and easy to use in the operating room. It was successfully tested on 25 patients treated for an infiltrative breast cancer. However, despite its small practical size, this detector's field of view turned out to be small and surgeons had to proceed acquiring up to 4 different images to cover the entire axillary area, which led to the idea of a 100 °— 100 mm2 gamma camera. This study reveals the details and the characteristics of our device shedding the light on its performances and the unique dimensions of its field of view covering the entire axillary area and the advantages of its use during surgical operations.

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MALIGNANT GROWTH IN THE BONE MARROW CREATES ABNORMAL HEMATOPOIETIC PROGENITOR CELL NICHES

Angela Colmone, Maria Amorim, Andrea Pontier, Sheng Wang, Elizabeth Jablonski, Dorothy A. Sipkins

Department of Medicine, Section of Hematology/Oncology, The Universityof Chicago, 5841 S Maryland Ave MC 2115, Chicago, IL, 60605 USA

The host tissue microenvironment is known to influence malignant cell proliferation and metastasis, but little is known about how tumor-induced changes in the microenvironment impact benign cellular ecosystems. Using dynamic in vivo imaging, we show that leukemic cell growth disrupts normal hematopoietic progenitor cell (HPC) bone marrow (BM) niches and creates abnormal microenvironments that sequester transplanted HPCs. HPCs in leukemic mice decline in number over time and fail to mobilize into the peripheral circulation in response to cytokine stimulation. Neutralization of SCF secreted by leukemic cells inhibits HPC migration into malignant niches, normalizes HPC numbers, and restores HPC mobilization in leukemic mice. Our data provide evidence that the tumor microenvironment can cause HPC dysfunction by usurping normal HPC niches, and that inhibiting HPC interaction with tumor niches may maintain normal progenitor cell function in the setting of malignancy.

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EPITHELIAL-MESENCHYMAL TRANSFORMATION IN SOLID TUMORS WITH EMPHASIS ON HEAD AND NECK SQUAMOUS CARCINOGENESIS

Adel K. El-Naggar

The University of Texas M.D.
Anderson Cancer Center, Houston, TX, USA

Phenotypic and conformational changes in the epithelial/host interface during development and progression of head and neck squamous carcinoma are unknown. However, it is generally believed as in other epithelial carcinomas, the cells undergo structural and morphologic modifications in response

to host cell interaction leading to Epithelial to Mesenchymal Transformation (EMT). This process appears to play a crucial role in tumor invasion, progression, and metastasis of squamous carcinogenesis. Identifying the key regulators of this process in HNSC may provide critical information on tumor host interactions in early and progressive stages of these tumors. Although, several pathways have been linked to the induction of EMT in several human carcinomas, differential involvement of specific pathways in each tumor type exist. The presentation will highlight the significance of EMT in early and invasive HNSC and discuss the potential therapeutic and biological implications.