european journal of histochemistry
ISSN 1121-760X
volume 55/supplement 1
2011

PROCEEDINGS OF THE 34th NATIONAL CONGRESS OF THE ITALIAN SOCIETY OF HISTOCHEMISTRY

San Benedetto del Tronto, June 7-9, 2011

Centro Congressi Palariviera

Università di Camerino

Presidente

Francesco Amenta

Vice Presidenti

Maria Gabriella Gabrielli, Daniele Tomassoni

under the auspices of
the University of Pavia, Italy

Published by PAGEPress, Pavia, Italy

Editorial Office:
via G. Belli, 4 27100 Pavia, Italy
Phone: +39.0382.1751762 - Fax: 39.0382.1750481
E-mail: info@pagepress.org

Printed quarterly by:
Tipografia PIME Editrice srl
via Vigentina 136
27100 PAVIA, Italy
Phone: +39.0382.572169 – Fax +39.0382.572102
E-mail: tipografia@pime-editrice.it
VAT no. 00280810185

Annual Subscriptions
Europe: Euro 160
All other Countries: $ 200

Subscriptions, cancellations, business correspondence and any enquiries must be sent to PAGEPress Publications, Pavia, Italy. Cancellations must be received before the end of September to take effect at the end of the same year.

Reg. Tribunale di Pavia n. 289/23.2.1984.

Supported by the Ministero per i Beni e le Attività Culturali, Italy as a publication of high cultural value.

Associato all'USPI
Unione Stampa Periodica Italiana

european journal of histochemistry
ISSN 1121-760X
volume 55/supplement 1
2011

ejh

under the auspices of
the University of Pavia, Italy

European Journal of Histochemistry

The European Journal of Histochemistry was founded in 1954 by Maffo Vialli and published untill 1979 under the title of Rivista di Istochimica Normale e Patologica, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is published under the auspices of the Università of Pavia and of the Ferrata Storti Foundation, Pavia, Italy.
The European Journal of Histochemistry is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC).

The Journal publishes Original papers, Technical reports, Reviews, Brief notes, Letters to the Editor, Book reviews, Views and Comments, concerning investigations performed with the aid of biophysical, biochemical, molecular-biological, enzymatic, immunohistochemical, cytometric, and image analysis techniques.
Areas of particular interest to the European Journal of Histochemistry include:
- functional cell and tissue biology in animals and plants;
- cell differentiation and death;
- cell-cell interaction and molecular trafficking;
- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.

34th NATIONAL CONGRESS OF THE ITALIAN SOCIETY OF HISTOCHEMISTRY

San Benedetto del Tronto, June 7-9, 2011
Centro Congressi Palariviera
Università di Camerino

ORGANIZING COMMITTEE

Francesco Amenta, Maria Gabriella Gabrielli, Daniela Accili, Daniele Tomassoni, Seyed Khosrow Tayebati

SCIENTIFIC SECRETARIAT

Daniele Tomassoni, Maria Gabriella Gabrielli

TECHNICAL SECRETARIAT

Rosella Cavalieri, Daniela Gentili, Isabel Pirillo

GUEST EDITORS

Francesco Amenta, Maria Gabriella Gabrielli, Daniele Tomassoni

table of contents

european journal
of histochemistry
ISSN 1121-760X
volume 55/supplement 1
2011

PROCEEDINGS OF THE 34th NATIONAL CONGRESS OF THE ITALIAN SOCIETY OF HISTOCHEMISTRY

San Benedetto del Tronto (AP), June 7-9, 2011

MAFFO VIALLI AWARD LECTURE

FROM CHEMICAL NEUROANATOMY TO FUNCTIONAL UNDERSTANDING OF THE OLFACTORY SYSTEM

L. Oboti, P. Peretto, S. De Marchis, A. Fasolo

Dept. Human and Animal Biology, University of Torino, Italy. E-mail: aldo.fasolo@unito.it

The olfactory system is a privileged model, well suitable for studying the development of neuro-endocrine circuits, neural plasticity and repair, adult neurogenesis, learning in meaningful behavioural contexts, neural network modelling. The understanding of this system was strongly supported by classical histochemistry. The case of the Olfactory Marker Protein (OMP) staining is emblematic. OMP was the first, powerful marker for fully differentiated olfactory neurons and a key tool to investigate the dynamic relations between peripheral sensory epithelia and central relay region, the olfactory bulb (OB). Similarly the use of thymidine-analogs was able to show neurogenesis in an adult mammalian brain far before modern virus labelling and lipophilic tracers based methods. Nowadays, a wealth of new histochemical techniques combining cell and molecular biology approaches is available, permitting to move the analysis from the chemically coded circuitries to functional significance. The study of adult neurogenesis is indeed one of the best explanatory examples of such statement. After defining cell types involved and basic rules of this phenomenon in OB plasticity, we can now go further toward the role of neurogenesis in well testable behaviours related to socio-chemical communication in rodents. Accordingly, neuronal turn-over seemingly has an important functional value in neuro-endocrine regulations.

(Financial supports from Compagnia di San Paolo, Torino; MIUR)

Symposium I
Cytochemistry of the Cell Nucleus
In memoriam prof. Maria Gabriella Manfredi Romanini

SYNTHESIS, MOVEMENT AND DESTRUCTION OF RNAs IN THE NUCLEUS

M. Costanzo,[1] B. Cisterna,[1] O.O. Zharskaya,[2] O.V. Zatsepina,[2] M. Biggiogera[1]

[1]Laboratory of Cell Biology and Neurobiology, University of Pavia, Pavia, Italy; [2]Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
E-mail: marco.biggiogera@unipv.it

The subcellular localization of nascent hnRNAs in the perichromatin region is well known; this cannot be said, however, for the sites where the degradation of RNAs takes place. Considering that, according to the literature, about 90% of newly synthesized RNA is almost immediately degraded in the nucleus, we were interested in localizing both the sites and the factors involved in this mechanism. To this purpose, we have studied by means of ultrastructural immunocytochemistry and EM *in situ* hybridization the subcellular localization of several nuclear and nucleolar factors involved in transcription of pre-rRNA and pre-mRNA. In particular, we have focused on the nuclear localization of RNase A in culture cells in control conditions, in aging cells and after drug treatments (anisomycin, DRB, actinomycin D, ATP blockade). We have found that roundish, lightly electron dense bodies can contain significant amounts of RNAse A, B23, Ag-NOR proteins, PML as well as low amounts of snRNPs and hnRNP core proteins. These bodies also contain RNA but not poly (A) RNA. Apparently, ribosomal subunit proteins are not present within these structures. Moreover, these bodies can be found in close vicinity of the nuclear envelope or nucleolar periphery and we suggest that they are mobile. Their role in transport of some factors involved in transcriptional and maturation mechanism seem to be clear, and stimulates the hypothesis of delivering the appropriate factors in loco, including RNase. The latter might be required for the process of removing part of the defective hnRNA present in the nucleus.

APOPTOTIC NUCLEUS DISCLOSURE BY MEANS OF CYTOCHEMICAL APPROACHES

E. Falcieri

Dept. Earth, Life and Environm. Sciences, Urbino Univ. "Carlo Bo" and IGM, CNR, IOR, Bologna, Italy

During apoptosis, cell chromatin undergoes characteristic morphological changes, which have been long described, even if not yet completed understood. A common pattern of apoptotic death is DNA cleavage, initially producing large portions (50Kbp), followed by nucleosomic fragments.[1] Nevertheless, apoptosis without DNA cleavage, has been reported. Therefore, cytochemical approaches appear the right mean to better highlight this variable nuclear behavior. Hemopoietic cells show a great sensitivity and represent the most common *in vitro* apoptotic model, but, recently, apoptotic role in a variety of diseases, raised a new interest on neurons, chondrocytes and muscle cells. Most cytochemical approaches to apoptotic nucleus have been long addressed to DNA behaviour. The use of osmium-ammine, highly specific for nucleic acids, evidences a progressive DNA concentration, in dense apoptotic chromatin and micronuclei.[2] Moreover, TUNEL and flow cytometry suggest that DNA is firstly cleaved and then condensed. A role of cytoskeleton, and, more specifically, nucleoskeleton, in this phenomenon was postulated. Immunocytochemistry revealed, in apoptotic HL60 cell nuclei, the presence of actin, generally absent in nonapoptotic cell nuclear matrix.[3] TUNEL is a powerful tool to identify double strand DNA fragmentation *in situ*, independently of DNA fragment sizes. A modified TUNEL method,[4] applied to electron microscopy, recently allowed to correlate DNA breaks to their localization within apoptotic nuclear domains. Cytochemical approaches can so correlate biochemical pathways to chromatin changes, so better highlighting the apoptotic scenario.

References

1. D'Emilio et al. Histol Histopathol 2010,25;21
2. Falcieri et al. Histochem 1994,102; 221.
3. Luchetti et al. Apoptosis 2002,7;143.
4. Burattini et al. Microsc Res Techn 2009,72;913

EEF1A PHOSPHORYLATION IN THE NUCLEUS OF INSULIN-STIMULATED C2C12 MYOBLASTS: SER[53] IS A NOVEL SUBSTRATE FOR PROTEIN KINASE CβI

M. Piazzi,[1,2] A. Bavelloni,[2] I. Faenza,[1] W. Blalock,[1] L. Cocco[1]

[1]Dept. of Anatomical Science, Cellular Signaling Laboratory, University of Bologna, Bologna, Italy; [2]Cell Biology and Electron Microscopy Laboratory, IOR, Bologna, Italy.
E-mail: lucio.cocco@unibo.it

Recent data indicate that some PKC isoforms are translocated to the nucleus, in response to certain stimuli, where they play an important role in nuclear signaling events. To identify novel interacting proteins of conventional PKC (cPKC) at the nuclear level during myogenesis and to find new PKC isozyme-specific phosphosubstrates, we performed a proteomics analysis of immunoprecipitated nuclear samples from mouse myoblast C2C12 cells following insulin administration. Using a phospho(Ser)-PKC substrate antibody, specific interacting proteins were identified by LC-MS/MS spectrometry. A total of 16 proteins with the exact and complete motif recognized by the phospho-cPKC substrate antibody were identified; among these, particular interest was given to eukaryotic elongation factor 1 (eEF1A). Nuclear eEF1A was focalized in the nucleoli, and its expression was observed to increase following insulin treatment. Of the cPKC isoforms, only PKC I was demonstrated to be expressed in the nucleus of C2C12 myocytes and to co-immunoprecipitate with eEF1A. In-depth analysis using site-directed mutagenesis revealed that PKC I could phosphorylate Ser[53] of the eEF1A2 isoform and that the association between eEF1A2 and PKC I was dependent on the phosphorylation status of eEF1A2.

References

1. Bavelloni A, et al. Proteomics 2006;6:5725-34.
2. Faenza I, et al., Endocrinology 2007;148:1108-17.
3. Cocco L, et al. Adv Enzyme Regul. 2006;46:2-11.
4. Piazzi M, Bavelloni A, Faenza I, Blalock W, Cocco L. Mol Cell Proteomics 2010;9:2719-28.

IL6 TREATMENT INDUCES MYOCARDIAL DIFFERENTIATION IN H9C2 CELLS THROUGH PKC-ZETA ACTIVATION

M.A. D'Amico,[1] P. Izzicupo,[1] A. Di Fonso,[1] A. Bascelli,[1] M. Di Mauro,[2] S. Gallina,[2] A. Di Baldassarre[1]

[1]University "G.d'Annunzio", Dept. of Biomorphology, Chieti, Italy; [2]University "G.d'Annunzio", Dept. of Human Movement Science, Chieti, Italy
E-mail: ma.damico@unich.it

The study of embryonic cells differentiation into cardiomyocytes appears to be useful to understand the molecular bases of regeneration, since precursor cells present in adult heart seem to differentiate through similar signaling pathways. Since IL6-130kDa glycoprotein (gp130)-signaling is involved in the remodeling processes of myocardial cells, influencing cell growth and differentiation and inducing protein kinaseC (PKC)-dependent apoptosis, aim of this study was to analyze the possible differentiating effects of IL-6 in embryonic cells. H9c2 cell line was cultured in low serum medium up to 3 days in presence of IL6 (10 ng/mL). Western Blot and Immunofluorence analysis demonstrated that 3 days of treatment induced a marked increase of α-myosin heavy chain expression, a terminal cardiac differentiation marker, a microfilament reorganization and morphological modifications, including cells elongation and fusion into multinucleated tubes; these events were accompanied by nuclear translocation of Nkx2.5, an early myocardial development transcription factor, and subcellular redistribution of gp130. The IL-6 differentiation effects were at least partially mediated by PKCζ activation, whose phosphorylation levels importantly increased after 30 min of IL6 treatment. No changes in PKCα and PKCδ phosphorylation levels were detected. These data indicate that IL6 treatment induces differentiation in cardiomyoblasts through PKC pathway activation, and suggest a possible involvement of IL6-dependent pathways in regenerative capacity of myocardial cells following injuries.

CANDIDATE STEM CELL NICHES IN THE PERIBILIARY GLANDS OF HUMAN BILE DUCTS

G. Carpino,[1,4] V. Cardinale,[2] P. Onori,[5] A. Franchitto,[1] R. Mancinelli,[1] P. Berloco,[3] M. Rossi,[3] L. Reid,[6] D. Alvaro,[2] E. Gaudio[1]

Depts. of [1]SAIMLAL, [2]Clinical Medicine and [3]General Surgery and Organ Transplantation, Sapienza University of Rome, Italy; [4]Dept. of Health Sciences, "Foro Italico" University, Rome, Italy; [5]Dept. of Experimental Medicine, University of L'Aquila, Italy; [6]University of North Carolina, Chapel Hill, NC, USA. E-mail: guido.carpino@uniroma4.it

Hepatic stem cells have been identified in the canals of Hering and in bile ductules.[1] Whether this niche supports the repopulation of large intra-hepatic bile ducts (IHBDs) and extra-hepatic bile ducts (EHBDs) is debated. Peribiliary glands (PBGs) are tubulo-alveolar glands in the deeper tissue of large IHBDs and EHBDs.[2] PBGs have been suggested as a site of multipotent stem cells of endodermal origin.[3] The aim of this study is to characterize their putative niche. Large intra-hepatic bile ducts (N=10) and extrahepatic biliary tree (N=10) have been evaluated by routine histology and immunohistochemistry for several markers of stem and mature cells. Our results indicated that, in PBGs, multipotent stem cells of endodermal origin could be found. These cells are mostly located at the bottom of the glands near the fibromuscolar layer of the duct wall and their number reduces moving towards the surface epithelium. PBGs contain few cells expressing markers of mature liver and endocrine pancreas. Our observation could have profound implications for regenerative medicine and in pathophysiology. The PBGs, as stem cell niches, could represent a new source for stem cells able to give rise to liver and endocrine pancreas; furthermore they could play a relevant role in injury repair other than as sites of origin of malignancies.

References

1 Gaudio E et al. Dig Liver Dis 2009, 41:455-62.
2. Nakanuma Y. Pathol Int 2010, 60: 419-429.
3. Cardinale V et al. J Hepatol 2010, 52 (S1): S368.

GESTATIONAL DIABETES MELLITUS INTERFERES WITH THE BIOLOGICAL CHARACTERISTICS OF WHARTON'S JELLY MESENCHYMAL STEM CELLS.

P. Lanuti,[1,2,3] L. Pierdomenico,[1,2,3] R. Lachmann,[1,2] G. Grifone,[4] E. Vitacolonna,[1,2] M. Marchisio,[1,2,3] S. Miscia[1,2,3]

[1]Section of Human Morphology, Department of Medicine and Aging Science (D.M.S.I.), University "G.d'Annunzio" of Chieti-Pescara, Chieti; [2]Center for Aging Sciences (Ce.S.I.), "Università G.d'Annunzio" Foundation, Chieti; [3]StemTeCh Group, Chieti; [4]Institute of Molecular Genetics, National Research Council (CNR) Chieti, Italy.

Recent research indicates that the origin of obesity and related metabolic disorders is not only caused by genetic and risk factors in adult life (unbalanced diet, insufficient physical activity) but also may be influenced by the perinatal environment. In addition, studies in animal models suggest that the mesenchymal stem cell commitment into pre-adipocytes can already occur during fetal development and perinatal life. Since the number of pre-adipocytes and mature adipocytes is lower in normal subjects than in obese subjects, changes in the prenatal maturational process may play a role in the pathogenesis of obesity and metabolic-associated diseases. Gestational diabetes mellitus is related to an increased risk of obesity, early onset of metabolic syndrome and type 2 diabetes in the offspring. For this reason it would be useful to investigate how the perinatal environment may affect fetal mesenchymal stem cells, especially in deregulated gestational diabetes, where the fetal environment is modified in terms of hormone levels and nutrition. Therefore, we have compared Wharton's jelly mesenchymal stem cells (WJ-MSC) obtained from umbilical cord of both healthy and diabetic mothers, in order to better understand the mechanisms involved in metabolic diseases in offspring of diabetic mothers. Results indicate that WJ-MSC from diabetic mothers display, in contrast to cells from healthy mothers, a higher ability to differentiate towards the adipogenic lineage. This suggests that the diabetic uterine environment may be responsible for a "pre-commitment" that could give rise in the post natal life to an alteration of adipocyte production upon an incorrect diet style, which in turn would produce obesity.

Symposium II
Developmental Competence of Female Gamete: Nuclear, Cytoplasmic and Ovarian Factors

A NOVEL ROLE FOR PROGESTERONE RECEPTOR MEMBRANE COMPONENT-1 (PGRMC1) IN REGULATING MITOSIS AND MEIOSIS

V. Lodde,[1,2] J.J. Peluso,[2] F. Franciosi,[1] A.M. Luciano[1]

[1]Dept. of Animal Sciences, University of Milan, Milano, Italy; [2]Dept. of Cell Biology, University of Connecticut Health Center, Farmington, CT, USA.
E-mail: valentina.lodde@unimi.it

PGRMC1 is a protein involved in the regulation of numerous biological functions. Recently it was detected among the proteins of the mitotic spindle, suggesting a role during cell division. Therefore, the present studies were designed to assess the role of PGRMC1 in regulating ovarian cell mitosis and oocyte meiosis. We first tested whether progesterone (P4) and PGRMC1 affect mitosis through a microtubule-dependent process using rat spontaneously immortalized granulosa cells (SIGCs) and human ovarian cancer cells, SKOV3 cells. Immunofluorescence revealed that PGRMC1 localizes to the spindle apparatus and to the centrosomes during mitosis. *In situ* proximity ligation assay revealed that PGRMC1 interacts with β-tubulin. We found that P4 increases the stability of the spindle microtubules in response to cooling. Further, studies in SKOV3 cells indicated that PGRMC1 mediates P4's action on the spindle microtubules. In a second set of experiments we tested whether PGRMC1 affects bovine oocyte meiosis. Confocal imaging demonstrated that in immature oocytes, PGRMC1 localizes throughout the nucleus. At metaphase I and II, PGRMC1 concentrates in the centromeres, while in ana-telophase I stages it concentrates between the separating chromosomes. A colocalization study also revealed that PGRMC1 associates with the phosphorylated form of Aurora kinase B. Finally, PGRMC1 antibody injection impaired oocytes capability to mature *in vitro*. Taken together these observations suggest a pivotal role for PGRMC1 in both ovarian cells mitosis and oocyte meiosis, which may be specifically related to the mechanism(s) by which chromosomes segregate.

ULTRASTRUCTURAL FEATURES AND MEIOTIC SPINDLE DYNAMICS IN HUMAN MATURE OOCYTES SUBJECTED TO DIFFERENT CRYOPRESERVATION PROTOCOLS

S.A. Nottola,[1] G. Macchiarelli[2]

[1]Dept. of Anatomy, Histology, Forensic Medicine and Orthopaedics, La Sapienza University, Rome; [2]Dept. of Health Sciences, L'Aquila University, L'Aquila, Italy.
E-mail: stefania.nottola@uniroma1.it

Oocyte cryopreservation protocols have not been fully optimized yet. Our aims were to evaluate and compare the subcellular features shown by human mature oocytes frozen-thawed (F/T) using different protocols of cryopreservation (slow cooling vs vitrification). The oocytes were fixed at sampling (fresh controls) and after freeze/thawing. Main morphological features, including size, shape and organelle distribution, were analyzed by light and transmission electron microscopy in fresh and F/T oocytes. Meiotic spindle integrity was evaluated by confocal microscopy. Both fresh and F/T oocytes were generally rounded, 90-100 microns in diameter, provided with an ooplasm showing a uniform distribution of organelles. A slight to moderate vacuolization was found in the cytoplasm of F/T oocytes subjected to slow cooling. On the contrary, vacuoles were only occasionally detected in F/T oocytes after vitrification, and in fresh controls as well. Amount and density of cortical granules (CGs) appeared abnormally reduced in F/T oocytes, irrespective of the protocol applied (slow cooling or vitrification). Finally, under both conditions, chromosome alignment on the meiotic spindle appeared partly compromised. In conclusion, a) the cryopreservation protocols currently in use ensure a good overall preservation of the oocyte; b) vacuolization appears as a recurrent form of cell damage during slow cooling, whereas the quasi absence of vacuoles seems the most relevant marker of quality in vitrified oocytes; c) premature CG exocytosis seems a non-specific, ubiquitous phenomenon occurring during freeze/thawing, suggesting the appropriateness of the use of ICSI as the preferred insemination method after cryopreservation; d) possible chromosomal misalignment in F/T oocytes may represent an indication for an increased risk of meiotic errors and further studies are needed in order to ascertain if this anomaly may be related to post-warm culture timing before insemination.

NEW INSIGHTS INTO THE MECHANISMS OF FERTILIZATION: COMPARISON OF THE FERTILIZATION STEPS, COMPOSITION, AND STRUCTURE OF THE ZONA PELLUCIDA BETWEEN HORSES AND PIGS

S. Mugnier,[1] M.E. Dell'Aquila,[2] J. Pelaez,[1] C. Douet,[1] B. Ambruosi,[2] T. De Santis,[2] G.M. Lacalandra,[2] C. Lebos,[3] P.-Y. Sizaret,[3] B. Delaleu,[1] P. Monget,[1] P. Mermillod,[1] M. Magistrini,[1] S. Meyers,[4] G. Goudet[1]

[1]INRA, UMR PRC, CNRS, Université de Tours, IFCE, Nouzilly, France; [2]Department of Animal Production, University of Bari, Valenzano, Bari, Italy; [3]Département des Microscopies, Université de Tours, Tours, France; [4]Department of Anatomy, Physiology and Cell Biology, University of California-Davis, Davis, California, USA.
E-mail: ghylene.goudet@tours.inra.fr

Most studies exploring the mechanism of fertilization have been restricted to a single species, generally the mouse, without a comparative approach. We develop a comparative strategy between two divergent models, because the identification of divergences between species could allow us to highlight key components in the mechanism of fertilization. The two models are the pig, which has high *in vitro* fertilization (IVF) and polyspermy rates, and the horse, which has low IVF rates and no polyspermy. We compared the ability of equine and porcine gametes for zona pellucida (ZP) binding, acrosome reaction, penetration through the ZP, gametes fusion and pronucleus formation, using intraspecific and interspecific IVF. We showed that the ZP is a determining element in sperm-ZP attachment and penetration, whereas the capacity of the spermatozoa is of less importance. In contrast, the capacity of the spermatozoa is a key component of the acrosome reaction step. We compared the composition and the structure of the equine and porcine ZP, and we observed differences in the number and localisation of the ZP glycoproteins and in the mesh-like structure of the ZP. These differences could be related to the differences in spermatozoal attachment and penetration rates.

ULTRASTRUCTURE AND DISTRIBUTIONAL ARRANGEMENT OF MITOCHONDRIA IN PREPUBERTAL AND ADULT SHEEP OOCYTES

M.G. Palmerini,[1] S.A. Nottola,[2] M. Maione,[2] C. Lorenzo,[1] S. Succu,[3] F. Berlinguer,[3] G.G. Leoni,[4] S. Naitana,[3] G. Macchiarelli[1]

[1]Dept. of Health Sciences, University of L'Aquila, L'Aquila; [2]Dept of Anatomy, Histology, Forensic Medicine and Orthopaedics, La Sapienza University, Rome; [3]Dept. of Animal Biology, University of Sassari, Sassari; [4]Dept. of Physiological, Biochemical and Cellular Science, University of Sassari, Sassari, Italy.
E-mail: mariagrazia.palmerini@cc.univaq.it

The objective of this study was to compare the *in vitro* developmental competence of prepubertal (one-month-old) and adult sheep oocytes in terms of the 1) ultrastructural morphology and 2) mitochondrial distribution. Cumulus-oocyte complexes undergo IVM in standard condition for 24 h. Following decumulation by pipetting, half of the mature metaphase II (MII) stage oocytes were fixed in 2.5% glutaraldehyde and processed for light and transmission electron microscopy (LM and TEM) observations. The other half were subjected to immunostaining with MitoTracker Red (MT-Red, to label mitochondria with functionally active membrane potential) and Hoechst 33342 and analyzed by confocal microscopy (CM). Immature germinal vesicle (GV) stage oocytes, retrieved at 0 h IVM, were used as controls. By LM and TEM all the oocytes were regularly rounded, covered by microvilli and surrounded by an intact zona pellucida. Numerous rounded, oval or hooded mitochondria appeared either isolated or arranged in clusters in the ooplasm. At 0 h IVM the GV was rounded in prepubertal oocytes, whereas in the adult it appeared often flattened against the oolemma, with a crescent-shaped outline interpretable as an early sign of resumption of meiosis. Scattered cortical granules (CGs) were rarely found in the ooplasm of both prepubertal and adult oocytes. After 24 h of IVM, CGs became abundant and distributed in a single row under the oolemma, particularly in adult oocytes. By CM analysis it was found a homogeneous fine-to-granular mitochondrial distribution in prepubertal GV and MII oocytes. Conversely, in adult GV oocytes the mitochondrial pattern appeared granular to become arranged in clusters in MII oocytes. In conclusion, both prepubertal and adult oocytes completed maturation after 24 h in culture and showed an overall good preservation following IVM. However, the diverse distribution of mitochondria in prepubertal oocytes could explain, at least in part, their scarce developmental competence.

IN VITRO DEVELOPMENT OF OOCYTES FROM PRIMORDIAL FOLLICLES

E.E. Telfer

Institute of Cell Biology and Centre for Integrative Physiology, The University of Edinburgh, Hugh Robson Building, George Square, Edinburgh, Scotland.
E-mail: etelfer@staffmail.ed.ac.uk

The ability to develop human oocytes from the earliest follicular stages through to maturation and fertilisation *in vitro* would revolutionise fertility preservation practice. This has been achieved in mouse where *in vitro* grown (IVG) oocytes from primordial follicles have resulted in the production of live offspring. However, developing IVG systems to support complete development of human oocytes has been more difficult because of differences in scale of timing and size. Successes in growing human oocytes *in vitro* are being made in a step wise manner and the challenge now is to obtain complete oocyte development *in vitro*. Our lab has been working on a multi step culture system to support growth and development of bovine and human oocytes from primordial through to fully grown using fresh and cryopreserved ovarian cortical tissue. Our recent work has shown that human and bovine primordial follicles can be activated *in vitro* within ovarian cortical pieces and grow to multilaminar preantral (secondary) stages within 6 days (Step 1). These preantral follicles can be isolated and have the potential to grow to the antral stage (Step 2) within a total culture period of 10 days. Further 18 days *in vitro* results in fully grown oocytes in the bovine model. This time scale makes the complete *in vitro* development of oocytes from human tissue a practical and viable prospect. This presentation will focus on the approaches being taken to obtain complete *in vitro* development of human oocytes and histochemical strategies for assessment of subsequent growth rate and health of IVG oocytes will be discussed.

GAP JUNCTION-MEDIATED INTERCELLULAR COUPLING CONTROLS CHROMATIN REMODELING DURING BOVINE OOCYTE GROWTH AND DIFFERENTIATION THROUGH cAMP-DEPENDENT MECHANISM(S)

A.M. Luciano, V. Lodde, F. Franciosi, I. Tessaro, D. Corbani, S. Modina

Department of Animal Sciences, University of Milan, Milano Italy. E-mail: alberto.luciano@unimi.it

Oocyte development is characterized by impressive changes in chromatin structure and function in germinal vesicle (GV), which are crucial to confer the oocyte with meiotic and developmental competences. During oogenesis, oocyte and follicular cells communicate by paracrine and junctional mechanisms. In cow, oocyte-cumulus complexes (COCs) isolated from early antral follicles have uncondensed chromatin (GV0), functional gap junction mediated communications (GJC) and a limited meiotic competence. Aim of this study was to analyze the role of GJC on chromatin remodeling process during the specific phase of folliculogenesis that coincides with the transcriptional silencing and sequential acquisition of meiotic and developmental capability. COCs were cultured in a FSH-based culture system that sustained GJC, promoted oocyte growth and the transition from GV0 to higher stage of condensation. When GJ functionality was experimentally interrupted, chromatin rapidly condensed and RNA synthesis suddenly ceased. These effects were prevented by the addition of Cilostamide, a phosphodiesterase 3 inhibitor, indicating that GJC's action on chromatin structure and function is mediated by cAMP. Treatment with Cilostamide during the culture further prolonged GJ coupling. Prolonging GJ coupling during oocyte culture prior to IVM enhanced the ability of early antral oocytes to undergo meiosis and early embryonic development. Altogether these evidences suggest that GJC between germinal and somatic compartment plays a fundamental role in the regulation of chromatin remodeling and transcription, which are in turn related to the competence acquisition, throughout cAMP dependent mechanism(s).

TOWARD ARTIFICIAL OVARY: ISOLATION AND CRYOPRESERVATION OF HUMAN PREANTRAL FOLLICLES

A. Camboni,[1] C.A. Amorim,[1] J. Vanacker,[1] V. Luyckx,[1] M.M. Dolmans,[1] A. Van Langendonckt,[1] J. Donnez[1]

[1]*Dept of Gynecology, Saint-Luc, Catholic University of Louvain, Brussels, Belgium.*
E-mail: alessandra.camboni@uclouvain.be

Transplantation of cryopreserved ovarian tissue constitutes an option for restoring fertility in young cancer patients. Unfortunately, the possibility of reintroducing tumour cells by ovarian tissue autografting cannot be excluded. To avoid this problem, primordial/primary follicles (PFs) may be isolated. Human PFs are usually isolated from frozen ovarian cortical strips, but uneven follicular density in ovarian cortex has a strong impact on the success of this technique. An alternative approach may be to cryopreserve PFs after isolation. However, no studies on human PF cryopreservation have ever been conducted. The aim of this study was therefore to test a protocol to slow-freeze isolated human PFs. Human ovarian biopsies from 4 women were processed for follicle isolation by Liberase enzymatic digestion. PFs were then embedded in an alginate matrix and some frozen using dimethyl sulfoxyde (DMSO) or ethylene glycol (EG) as cryoprotectants. Fresh and cryopreserved isolated PFs were cultured for 7 days. At different time points (after isolation, cryopreservation and IVC), follicular diameter was measured and follicles were evaluated by live/dead assays using calcein AM/ethidium homodimer-I fluorescent probes and then fixed for transmission electron microscopy (TEM). After enzymatic isolation, all the follicles were alive. After warming, the survival rate was 93.2% in DMSO group and 63.9% in EG group. At the end of culture, Fresh group showed a survival rate of 82%, DMSO group of 85.1% and EG group of 66.2%. After 7 days of IVC, a significant increase in follicular size was observed in Fresh and DMSO groups, but not in the EG group. By TEM, preliminary results showed a preserved morphology in both oocyte and follicular compartments in the majority of follicles in fresh and DMSO groups. Our results show that isolated human PFs may be cryopreserved using DMSO as a cryoprotectant, and then cultured for 7 days, without their viability, morphology or growth capacity being affected.

Symposium III
Heterogeneity and Biological Roles of Cell Glycoconjugates

CARBOHYDRATE ANTIGENS IN COLON CANCER

F. Dall'Olio, N. Malagolini, M. Chiricolo

Dept. of Experimental Pathology, University of Bologna, Italy. E-mail: fabio.dallolio@unibo.it

The carbohydrate molecules on the cell surface fine tune the interactions of the cells with their microenvironment. An aberrant glycosylation pattern, leading to the expression of tumor-associated carbohydrate antigens (TACA), is an hallmark of cancer tissues. TACA are important because they contribute to determine the aggressive phenotype of cancer cells and because they are cancer markers with diagnostic and prognostic value. Two TACA expressed by colon cancer tissues are particularly interesting: 1) the α2,6-sialylated lactosaminic chains of glycoproteins (Sia6LacNAc) and 2) the sialyl Lewis x (sLex) antigen. Sia6LacNAc expression is regulated by the cognate glycosyltransferase ST6Gal.1[1,2] and is recognized by the lectin from *Sambucus nigra* (SNA).[3] High SNA reactivity by colon cancer tissues is inversely associated with 5-years survival.[4] Forced over-expression of ST6Gal.1 in colon cancer cell lines modifies the phenotype of cancer cells.[5] When sLex is ectopically expressed by cancer cells, it mediates metastasis formation by allowing the binding of colon cancer cells with E- and P-selectin expressed by endothelial cells. We have shown that fucosyltransferase-VI (Fuc-TVI) is mainly responsible for sLex biosynthesis in both normal and cancer colon,[6] although it is not responsible for its over-expression in cancer. Experimental[7] and clinical data indicate that the low expression of sLex in normal colonic mucosa might be due to the concomitant high expression of an alternative carbohydrate structure.

References

1. Dall'Olio F et al. Int J Cancer 1989;44:434-9.
2. Dall'Olio F et al. Int J Cancer 2000;88:58-65.
3. Sata T et al. Am J Pathol 1991;139:1435-48.
4. Vierbuchen MJ et al. Cancer 1995;76:727-35.
5. Chiricolo M et al. Glycobiology 2006;16:146-54.
6. Trinchera M et al. Int J Biochem Cell Biol 2011;43: 130-9.
7. Malagolini N et al. Glycobiology 2007;17:688-97.

BRINGING PROTEOGLYCANS FROM THE BASIC LABORATORY RESEARCH TO THE CANCER PATIENT

R. Perris

COMT – Centre for Moolecular and Translational Oncology, University of Parma, Parma, Italy
E-mail: rperris@cro.it

Proteoglycans (PGs), a growing family of complex macromolecules, are fundamental regulators of most cellular functions and are believed to be strongly implicated in the alterations of these functions in neoplastic cells. The unparalleled structural-functional diversity of PGs endows them with the ability to serve as critical mediators of the tumour cells' interaction with the host microenvironment, as well as to contribute as key elements for the organization and dynamic remodeling of this milieu. Despite their undisputable importance during embryonic development, their pivotal homeostatic role in the adult organism, and their frequent misregulation in tumour lesions, the precise impact of PGs on tumorigenesis needs to be better resolved. Particularly challenging seems to ascertain to what extent selected PGs may catalyze tumour progression

versus to what extent they may inhibit it. In fact, recent observations highlighting antithetic roles of individual PGs suggest that some may act as putative "tumor suppressors", whereas others may serve as valuable prognostic/predictive biomarkers. From published and our own observations we can infer that discrete cell surface profiles of PGs may define a "metastatic signature" of a cancer cells. More integrated efforts are now needed to consolidate their routine use for the clinical monitoring of cancer patients and their exploitation potential as therapeutic targets. Several PGs have the required attributes for being considered as effective antigens for antibody-based anti-cancer therapies and the tangible results obtained in recent clinical trials targeting the NG2 transmembrane PG incite the further pursuing of studies aimed at transferring our knowledge about the role of PGs in tumour progression to alternative treatment modalities to apply to cancer patients.

GLYCOCALIX CHANGES IN MORPHO-FUNCTIONAL ALTERATIONS OF THE GLOMERULAR FILTRATION BARRIER DURING SEPSIS

E. Sgambati,[1] L. Bonaccini,[2] L. Vitali,[3] V. Selmi,[3] M. Margheri,[2] A. Tani[2]

[1]Dept. of Sciences and Technologies for Environment, University of Molise; [2]Dept. of Anatomy Histology and Forensic Medicine, University of Florence; [3]Dept. of Critical Care Medicine, University of Florence, Italy.
E-mail: eleonora.sgambati@unimol.it

Syndecan-1 and sialic acids are glycocalix components that contribute to maintain the structure and functions of the glomerular filtration barrier (GFB). The aim of this investigation was to evaluate expression changes of syndecan-1 and sialic acids in GFB, in early stages of an experimental animal model of polymicrobial sepsis. Experiments were performed on adult male rats assigned to two groups: 1) sham-operated (n=15); 2) Caecal Ligation and Puncture (CLP) (n=19). In order to evaluate sepsis, TNF-α levels in plasma and growth of microorganisms in the peritoneal fluid were relieved at 0 h, 3 h and 7 h after CLP or sham-operation. Kidney specimens were collected and structural and ultrastructural alterations in the GFB were assessed. Syndecan-1 expression was investigated by using immunofluorescence; sialic acids expression was evaluated by using lectin histochemistry (MAA, SNA, PNA and SBA), in association with enzymatic and chemical treatments. Protein urine level was measured to assess changes in GFB permeability. The results showed in septic rats: 1) a rise in TNF-α and growth of microorganisms in the peritoneal fluid; 2) massive proteinuria; 3) structural and ultrastructural changes of GFB; 4) significant decrease of syndecan-1 and decrease and change in chemical structure of sialic acids, in particular an increase of acetylation. These findings indicate that in sepsis, from its earliest development, alteration of GFB structure and ultrastructure, as such as of glycocalyx, occurs. This damage is associated to loss of GBF permeselectivity.

GLYCOHISTOGENESIS OF SHEEP ENZOOTIC NASAL TUMOUR

P. Scocco,[1] F. Mercati,[2] E. Lepri,[2] G. Vitellozzi,[2] P. Ceccarelli[2]

[1]School of Environ. Sci., University of Camerino, Camerino; [2]Dept. Biopathol. Sci. and Hygiene anim/ food prod., University of Perugia, Perugia, Italy.
E-mail: paola.scocco@unicam.it

This work is aimed to the glycohistochemical characterization of the sheep enzootic nasal tumour (ENT)[1] and to correlate it with the glucidic profile of normal nasal mucosa in order to hypothesize the histological origin of the tumour. ENT and contiguous macroscopically normal nasal mucosa from ethmoidal turbinates were collected from 2 adult Lacaune sheep that were euthanized due to chronic wasting disease with nasal discharge, dyspnoea and sneezing. Normal nasal mucosa were also removed from 5 healthy sheep. Collected tissues were treated for both conventional and lectin histochemistry. Normal samples from healthy and ill sheep showed the same reactivity. ENT is composed by papillary and tubular components. The papillary portion produces both secretion and surface glycoconjugates, represented by sulphated glycosaminoglycans and by neutral and sialilated glycoproteins. The tubular portion expresses neutral and sialilated glycoproteins as surface glycoconjugates. Most noticeable data is the expression by the both ENT growth patterns of C4 acetylated sialoderivatives which could confer a resistance towards pathogen agents to the tumour cells, while C4 acetylated sialic acids are lacking in normal nasal mucosa. On the basis of some analogies, such as the prevailing expression of secreting glycoconjugates like highly sulphated glycosaminoglycans and acid glycoproteins, we hypothesize the origin of the ENT papillary portion from the respiratory glands with a possible involvement of the goblet cells. Other analogies, such as the prevailing expression of neutral surface glycoproteins, allow us to suppose the tubular portion belonging from the olfactory glands.

Reference

1. De las Heras M et al. Eur J Vet Pathol 1998;4:1-5.

EFFECT OF HORMONAL SUPEROVULATION ON THE LECTIN BINDING PATTERN OF THE OVINE OVIDUCT

S. Desantis, G. Ventriglia, G. Accogli, F. Silvestre, F. Binetti, G.M. Lacalandra

Dept. of Animal Production, University of Bari Aldo Moro, Italy. E-mail: s.desantis@veterinaria.uniba.it

The oviduct plays an essential role in the mammalian reproduction and it undergoes significant endocrine-induced morphological, biochemical and physiological changes during the oestrous cycle. The functions of the oviduct epithelium are controlled by the ovarian steroids, oestrogen and progesterone.[1] In this study the glycoconjugate pattern of oviduct obtained from hormonally (FSH-P and eCG) superovulated ewes for oocytes recovery was analyzed. Oviducts from treated and control sheep were collected by laparatomy, fixed in 4% (w/v) neutral formalin, embedded in paraffin wax and sections processed for lectin histochemistry. In the ampulla, the luminal surface of all specimens showed strong reactivity with MAL II,SNA, PNA after KOH-sialidase (s) treatment, RCA_{120}, HPA, SBA, KOH-s-WGA, and GSA I-B4, whereas it stained strongly with GSA II, UEA I, and LTA in treated sheep which showed reactivity with KOH-s-PNA, SBA, GSA I-B4, GSA II also in the apical cytoplasm of non-ciliated cells. In the isthmus, the luminal surface showed same staining reactivity with RCA_{120}, SBA, and GSA I-B4 in all specimens, and a stronger affinity for MAL II, UEA I and LTA in treated ones. A distinctive feature of hormonized isthmus was the binding of the entire cytoplasm of ciliated cells and non-ciliated cells with MAL II, SNA, RCA_{120}, SBA, GSA II, UEA I, and LTA. These results indicate that ampulla and isthmus of ovine oviduct express a different glycoconjugate pattern and that the hormone administration for superovulation produces different effect along the oviduct. These differences could be related to the different functions of each segment that constitutes the ovine oviduct.

Reference

1. Buhi WC Reproduction 2002, 123:355-62.

MODULATION OF GLUCIDIC PROFILING IN THE INTESTINAL MUCOSA OF THE OBESE ZUCKER RATS

M.G. Gabrielli,[1] D. Tomassoni,[1] S. Panarello[1]
M.F Lokhandwala,[2] D. Accili[1]

[1]School of Biosciences and Biotechnology, University of Camerino, Camerino, Italy; [2]Heart and Kidney Institute, College of Pharmacy, University of Houston, Houston, TX, USA.

In the intestinal ecosystem, glycoconjugates expressed by the enterocytes and secreted by the goblet cells contribute to the first defensive barrier against pathogenic bacteria and luminal contents. Changes in goblet cell secretion and glycosylation of the intestinal glycocomponents were reported in a variety of intestinal and nutritional disorders and diseases, as well as in conditions requiring total parenteral nutrition. On the other hand, recent findings suggest a possible role for the gut microbiota in obesity and, consequently, in other aspects of metabolic syndrome. Based on these data, the present study was aimed to elucidate the expression and distribution of sialylated and fucosylated glycomponents in the intestinal mucosa of obese Zucker rats (OZR). OZR represent a model of type 2 diabetes exhibiting a moderate degree of arterial hypertension and increased oxidative stress. The occurrence of sialic acids differently linked to D-Gal and/or to D-GalNAc was demonstrated by SNA and MAL II lectin binding. Moreover, additional and complementary data on sialic acid acetylation degree and sites were provided by combining PNA and DBA lectins with chemical and enzymatic pretreatments. Application of LTA, UEA, and AAL lectins allowed to elucidate the fucose profiling in the intestinal epithelium and/or in its secretory products. The binding patterns obtained showed an overall lower intensity of lectin reactivity in OZR compared with control animals. Both sialic acid (α2,6)-D-Gal/D-GalNAc, sialic acid (α2,3)-D-Gal sequences, and fucose residues were affected suggesting possible correlations between the modified glucidic profiling, the obesity condition, and the characteristics of microbial modulation reported in metabolic syndrome.

Symposium IV
Plasticity of the Cerebral Cholinergic System
Joint Symposium with the XXI Meeting of the Gruppo Italiano per lo Studio della Neuromorfologia (G.I.S.N.)

THE BRAIN CHOLINERGIC SYSTEM: FROM NEUROBIOLOGY TO THERAPY

G. Pepeu

Dept. of Pharmacology, University of Florence, Florence, Italy. E-mail: giancarlo.pepeu@unifi.it

The brain cholinergic system is formed by neurons located in the cholinergic nuclei of the brain stem and midbrain, in the forebrain cholinergic nuclei, and by the striatal interneurons. The brain stem and midbrain neurons project mainly to the thalamus, hypothalamus and the forebrain nuclei. These in turn project to the cerebral cortex, hippocampus, amygdala and olfactory bulb forming a diffuse cholinergic network. The cholinergic neurons are characterized by specific features: cholinoacetyltrasferase coupled to a high affinity choline uptake mechanism for synthesizing acetylcholine (ACh), the presynaptic vesicles storing it, and the dependence on NGF. ACh released in the synaptic cleft acts on different subtypes of muscarinic and neuronal nicotinic receptors and is inactivated by acetylcholinesterase and butirylcholinesterase. Activation of the central cholinergic neurons induces arousal and plays a role in attention and memory formation. Striatal cholinergic neurons regulate motor activity. The brain cholinergic neurons undergo moderate degenerative changes during aging and mild cognitive impairment, and severe degeneration in Alzheimer's disease (AD) and other neurodegenerative diseases including alcoholic dementia and Parkinson's disease. The cholinergic hypofunction associated with neurodegenerative disease and schizophrenia, as well as that induced by cholinergic receptor blockade, result in cognitive impairment. Attempts to treat the cognitive deficits in AD and schizophrenia, and to improve memory in aging, have been made in the last 30 years by using cholinesterase inhibitors (ChEIs), receptor agonists, choline precursors, and by increasing NGF levels. Consistent, albeit temporary, results have been only obtained with ChEIs, and new nicotinic agonists seem promising.

DEGENERATIVE DEMENTIAS. CLINICS, NEUROPSYCHOLOGY AND NEUROTRANSMITTER SYSTEMS

A.M. Fasanaro, G. Colucci, S. Carpi

Alzheimer's Evaluation Unit and Involutive Brain Diseases, National Survey of Hospital A. Cardarelli, Naples, Italy. E-mail: giola.fasanaro@virgilio.it

Dementia is becoming a common disease in the Western word and the number of cases is estimated to increase in the next years. Alzheimer's disease is the most common form of dementia in the elderly, accounting for 50-56% of cases diagnosed clinically or at autopsy. Alzheimer's disease is characterized by deterioration of memory and other cognitive domains, and leading to death 3-9 years after diagnosis. It is important for clinicians to recognize early signs and symptoms of dementia and to identify modifiable risk factors and early disease markers. Identification of neurotransmitter systems most affected in single patients may contribute to establish the most appropriate treatments. This identification is possible using

sophisticated imaging techniques or to some extent with carefully done neuropsychological evaluation. This work summarizes the main clinical and neuropsychological correlations of neurotransmitter systems involvement in adult-onset dementia disorders and how this may be used for proper pharmacotherapeutic approaches.

NEUROIMAGING AND DIAGNOSIS OF ADULT-ONSET DEMENTIAS

F. Zeccolini

Unit of Neuroradiology, National Survey of Hospital A. Cardarelli, Naples. E-mail: fabio.zeccolini@fastwebnet.it

Dementia is the generic term for diseases in which memory problems are associated with the failure of other mental functions as to make the person dependent. Alzheimer's disease is the most common form of dementia (50%). Vascular dementia is also very common (18%). They meet often combined forms. The age is the greatest risk of contracting the disease. However, memory problems are not always a symptom of an early dementia. The mental faculties are altered with age, the rate of assimilation of information is slower and it affects the learning ability and memory. This is the reason why older people are apt to forget and think they are affected by the onset of dementia. Through neuropsychological testing and imaging, but are unable to distinguish clearly between memory disorders associated with aging, by the onset of dementia. Vascular dementia is the second level of frequency (18%). It is caused by arteriosclerosis of the blood vessels of the brain, which leads to a slowing of movement. This results in the death of tiny areas of the brain when it comes to micro-infarctions or entire regions when it comes to major disturbances of the circulation (cerebral infarction). MRI is the method of micro-level to distinguish many heart attacks that might otherwise go unnoticed. Main symptoms: sudden appearance of cognitive disorders related to vascular problems, mood swings, erratic evolution and gradual worsening of the disease. Currently, neuroradiology plays an important role in supporting the diagnosis of dementia. Over the last decade there has been a great increase in the number of published studies applying neuroimaging techniques to the study of degenerative dementia, particularly Alzheimer's disease.

PRESYNAPTIC MODULATION OF CHOLINERGIC NEUROTRANSMISSION

M. Marchi,[1,2,3] S. Zappettini,[1] A. Salamone,[1] G. Olivero,[1] M. Grilli[1]

[1]Dept. of Experimental Medicine, Section of Pharmacology and Toxicology, University of Genoa; [2]Center of Excellence for Biomedical Research, University of Genoa, Italy [3]National Institute of Neuroscience, Italy. E-mail: marchi@pharmatox.unige.it

Presynaptic muscarinic and nicotinic acetylcholine receptors in the CNS are present on cholinergic and non cholinergic nerve terminals. They may interact with other metabotropic or ionotropic receptors and produce integrated responses which, in turn, generates antagonistic or synergistic effects. The cross-talk between receptors represents an important mechanism of neurotransmission modulation and plasticity. It can occur by direct physical interactions as in the case of G protein-coupled receptor heterodimerization, or it may involve intracellular pathways. The facilitatory or inhibitory action of one receptor might therefore depend on the function of the other receptors co-existing on the neuron. Recent studies have shown that this phenomenon also concerns the muscarinic and the nicotinic receptor subtypes.[1] The understanding of these interactions may allow a better evaluation not only the pharmacological effects of cholinergic drugs but also the normal physiological role of the natural neurotransmitter acetylcholine. This presentation will focus on the co-existence and the functional interaction between the release regulating presynaptic nicotinic or muscarinic receptors and other receptors co-existing on the same axon terminals.

Reference

1. Marchi M. and Grilli M., Progr Neurobiol 2010;92:105-11.

PRECLINICAL EVIDENCE OF AN ASSOCIATION WITH CHOLINERGIC PRECURSORS AND CHOLINESTERASE INHIBITORS IN AN ANIMAL MODEL OF CEREBRAL VASCULAR DAMAGE

S.K. Tayebati,[1] D. Tomassoni,[2] I.E. Nwankow,[1] F. Amenta[1]

[1]Human Anatomy, School of Pharmacy; [2]School of Biosciences and Biotechnology, University of Camerino, Camerino, Italy. E-mail: khosrow.tayebati@unicam.it

Cholinergic hypofunction is a trait of Alzheimer's disease (AD)and of other forms of dementias such as vascular dementia (VaD) and Lewy bodies dementia (LBD). The acetylcholinesterase (AChE)/cholinesterase (ChE) inhibitors (Is) are one of the two classes of drug approved for AD treatment. ChE-Is are licensed for the symptomatic relief of mild to moderate AD. These drugs are used out of label as a therapeutic approach for treating cognitive disorders other than AD such as VaD and LBD. A main problem deriving from their use is that the benefits of AD symptomatic treatment are modest and not long lasting. It is also thought that the magnitude of benefit of this class of drugs can elicit is apparently marginal and difficult to detect and to measure clinically. Moreover, widespread use of AChE/ChE inhibitors may be accompanied by serious adverse events. Cholinergic precursors represent an old approach to treat cholinergic dysfunction, but the first drugs proposed did not show clinical benefit on symptoms of AD. Actually, evidence for an enhancement of ACh biosynthesis by choline or lecithin is faint. The same is not true for cytidine 5'-diphosphocholine and choline alphoscerate for which a modest improvement of cognitive dysfunction in dementia disorders is documented. The association of ChE-Is with phospholipids involved in choline biosynthetic pathways was proposed to further enhance cholinergic neurotransmission compared to single compounds. It could represent a strategy to provide a stronger cholinergic challenge in dementia. This study reviews the cholinergic hypothesis of geriatric memory dysfunction and discusses based on original finding the neurochemical bases of the association between ChE-Is and cholinergic precursors. Evidence of a possible neuroprotective effect of the association in animal models is also presented.

ASSOCIATION BETWEEN THE CHOLINESTERASE INHIBITOR DONEPEZIL AND THE CHOLINERGIC PRECURSOR CHOLINE ALPHOSCERATE IN ALZHEIMER'S DISEASE WITH ASSOCIATED CEREBROVASCULAR INJURY. FIRST RESULTS OF THE ASCOMALVA TRIAL

F. Amenta,[1] A. Carotenuto,[1] A.M. Fasanaro,[2] A. Lanari,[3] R. Rea,[1] E. Traini[1]

[1]Clinical Research Unit, School of Pharmacy,University of Camerino, Camerino; [2]Alzheimer's Evaluation Unit and Involutive Brain Diseases, National Survey of Hospital A. Cardarelli, Naples; [3]Unit of Neurology, Hospital C. Poma Mantova, Italy. E-mail: francesco.amenta@unicam.it

Here we summarize the first results of the ongoing clinical trial on the *Effect of association between a cholinesterase inhibitor (ChE-I) and choline alphoscerate on cognitive deficits in Alzheimer's disease associated with cerebrovascular impairment* (ASCOMALVA). This trial wants to assess if association between the ChE-I donepezil at the daily dose of 10 mg and choline alphoscerate at the daily dose of 1,200 mg/day was accompanied by changes in Mini Mental State Evaluation (MMSE), Basic Activities of Daily Living (BADL), Instrumental Activities of Daily Living (IADL) and Neuropsychiatric Inventory (NPI). This latter included evaluation of severity and of caregiver stress measures (NPIF and NPIS). At the moment this double-blind trial has completed the 6 months observation of 70 patients of the 210 planned . Patients were aged between 56 and 86 years (mean 75±10 years) and were included in the protocol with a MMSE score between 15 and 23. Patients should suffer from ischemic brain damage documented by neuroimaging (MRI and CT scan), with a score ≥2 in at least one subfield (white matter or basal ganglia) according to the new rating scale for age-related white matter changes (ARWMC). Recruited patients were then randomly allotted to an active treatment group (donepezil + choline alphoscerate) or to a reference treatment group (donepezil + placebo). The 70 patients so far analyzed were treated for 6 months and were examined at recruitement and after 3 and 6 months of treatment. Protocol plans to prolong treatment for 24 months and to check patients at 3, 6, 9, 12, 18 and 24 months after being enrolled in the trial. Patients allotted to the reference treatment group showed a slight time-dependent worsening of MMSE, IADL and NPIS scores and no changes in the BADL and NPIF scores were noticeable. Treatment with donepezil plus choline alphoscerate improved compared to donepezil alone the different items analyzed except the BADL that was slightly worsened. The above results suggest that association of the cholinergic precursor choline alphoscerate to the standard treatment with a ChE-I may represent a therapeutic option to prolong beneficial effects of cholinergic therapies in Alzheimer's disease patients with concomitant ischemic cerebrovascular disease.

Symposium V
Applied Histochemistry

COLONIC SMOOTH MUSCLE CELLS AND INTERSTITIAL CELLS OF CAJAL IN DIVERTICULAR DISEASE

B. Battolla,[1] L. Mattii,[1] C. Segnani,[1] C. Ippolito,[1] M.S. Faussone-Pellegrini,[2] R. Colucci,[3] C. Blandizzi,[3] P. Buccianti,[4] S. Dini,[1] A. Dolfi,[1] N. Bernardini[1]

Dept. of [1]Human Morphology and Applied Biology (Section of Histology), [3]Internal Medicine (Div. of Pharmacology and Chemotherapy), and [4]Surgery, University of Pisa; [2]Anatomy, Histology and Forensic Medicine, University of Florence; Italy. E-mail: nunzia.bernardini@med.unipi.it

Intestinal motility is regulated by complex interactions among smooth muscle cells (SMC) of muscularis propria, enteric nerve endings and interstitial cells of Cajal (ICC). In colonic SMC, transmenbrane channels rich in connexin (Cx), in particular Cx43, contribute to intercellular gap junctions, which ensure coordinated motor responses to nerve inputs. ICC are pacemaker cells which modulate neuroenteric transmission by connecting SMC with varicosities of myenteric neuron axons. There is evidence that SMC motility and gap junction permeability are regulated by the GTPase RhoA, an emerging key modulator of SMC phenotype. The purpose of the present study was to evaluate the patterns of Cx43 and RhoA expression in SMC, as well as ICC density in colonic specimens from patients affected by diverticular disease (DD), a condition associated with colonic motor dysfunction. The muscularis propria of surgical whole thickness colonic samples from DD patients was examined immunohistochemically for the expression of Cx43 and RhoA in SMC, and c-kit in ICC. Semiquantitative analysis of DD colonic specimens displayed a marked decrease in Cx43 and RhoA expression in SMC. There was also a reduced ICC density in myenteric ganglia (ICC-MY), circular (ICC-CM) and longitudinal (ICC-LM) muscle, as compared to controls. Overall, it is suggested that abnormalities in gap junctions and RhoA expression in SMC, together with a reduced density of muscular ICC, account for the colonic dismotility occurring in DD.

THE NEUROMUSCULAR COMPARTMENT IN INTESTINAL DYSMOTILITY: STATE OF THE ART

C. Segnani,[1] C. Ippolito,[1] B. Battolla,[1] M.S. Faussone-Pellegrini,[2] C. Blandizzi,[3] R. De Giorgio,[4] A. Dolfi,[1] N. Bernardini[1]

Dept. of [1]Human Morphology and Applied Biology (Section of Histology and Medical Embryology) and [3]Internal Medicine (Div. of Pharmacology and Chemotherapy), University of Pisa; [2]Anatomy, Histology and Forensic Medicine, University of Florence; [4]Clinical Medicine, University of Bologna; Italy.
E-mail: nunzia.bernardini@med.unipi.it

The disorders of gastrointestinal motility comprise a heterogeneous group of chronic conditions, which are considered to be relevant for public health, since they are very common, can be disabling, and induce major social and economic burdens. Abnormal bowel motility is associated with a wide range of diseases, differing for etiopathogenic mechanisms, pathologic lesions, and region of gut involvement (e.g., irritable bowel syndrome, slow transit constipation, inflammatory bowel disease, diverticular disease). These motor disturbances are suggestive of alterations of enteric neuromuscular cellular components, including cells of the enteric nervous system (i.e. myenteric neu-

rons, glial cells, interstitial cells of Cajal), and circular and longitudinal smooth muscle cells. Although the presence of intestinal dysmotility in patients with the above gut disorders has been well established in the clinical setting, scarce attention has been paid to the respective morphological arrangements of enteric cellular networks in the neuromuscular compartment of gut wall. In this regard, previous attempts, made to obtain reliable quantitative estimations of enteric cells involved in gut motility, yielded hardly comparable, or even conflicting, results. Thus, in order to overcome the lack or heterogeneity of current data, careful morphological examinations and development of standardized procedures are particularly required in the field of gastrointestinal neuromuscular pathology, as recently suggested (Knowles *et al*., Acta Neuropathol 2009; Knowles *et al*., Gut 2010).

UP-REGULATION OF ESTROGEN RECEPTOR ALPHA IN HUMAN THYROID PAPILLARY CARCINOMA: A POSSIBLE REGULATION BY HYPOXIA

M. Di Vito,[1] E. De Santis,[2] G.A. Perrone,[4] E. Mari,[1] L. Coppola,[4] G. Fadda,[5] M. Tafani,[1] L. Fumagalli,[2] M.A. Russo[1,3]

[1]Department of Experimental Medicine and [2]Department of Human Anatomy, Sapienza University, Rome; [3]Laboratory of Cellular and Molecular Pathology, IRCCS San Raffaele Pisana, Rome; [4]UOC Anatomia Patologica, Ospedale San Filippo Neri, Rome; [5]Institute of Pathologic Anatomy, Catholic University of Rome; Italy.
E-mail: elena.desantis@uniroma1.it

Estrogen (E2) is known to promote proliferation of thyroid papillary carcinoma cells. E2 regulate cell proliferation by binding to specific receptors: estrogen receptors (ERs). ERs include two isoforms: alpha (ER-A) having a proliferative and antiapoptotic activity and ER beta (ER-B) displaying differentiative and proapoptotic effects. The expression pattern of ER isoforms in normal and tumor thyroid tissues is still controversial and poor defined. Most of the discrepancies referred by many authors may be due to the method utilized: immunohistochemistry (IHC) appears to be inadequate for reliable quantitative studies whereas, molecular biology have generated highly variable data due to the cellular heterogeneity of primary tissue samples. Therefore, we studied the expression of ER isoforms using laser capture microdissection (LCMD), an innovative technique to isolate homogeneous, morphologically identified cell populations, followed by RT-PCR and Western Blot. Our results demonstrate that ER-A mRNA and protein is constantly overexpressed (200-300 folds) in cancer cells microdissected from human thyroid papillary ca, as compared with normal cells obtained from surrounding tissue. Moreover, ER-A expression is increased in human cancer cell line by hypoxia, commonly observed during tumor development, suggesting an involvement of ER-alpha in cellular pathways stimulating tumor cells survival.

INFLUENCE OF PHENOL RED ON PROTEIN KINASE C ALPHA MEDIATED STRESS RESPONSE TO HEMA IN HUMAN FEMALE GINGIVAL FIBROBLASTS

V. di Giacomo,[1,2] S. Zara,[1,2] M. Rapino,[3] A. Cataldi[1,2]

[1]Dept. of Medicine and Science of Aging,[2]Section of Human Anatomy, Faculty of Pharmacy, University G. d'Annunzio, Chieti-Pescara; [3]CNR Institute of Molecular Genetics, Unit of Chieti, Chieti, Italy. E-mail: cataldi@unich.it

Objectives. 2-Hydroxyethyl methacrylate (HEMA), component of dentin-bonding systems, by diffusing into oral cavity induces a cytotoxic response.[1] The interaction of HEMA with cell membrane determines Reactive Oxygen Species (ROS) production at mitochondrial level activating specific signaling pathways, including Protein Kinases C (PKC). Since a regulation is exerted by various PKCs on Nitric Oxide Synthase (NOS) activation, our aim was to investigate the role of PKCs and the possible interplay with upstream (ROS) and downstream (NO) signaling compounds in human female gingival fibroblasts (HGF) response to HEMA.[2] *Materials and Methods.* Cultured human female gingival fibroblasts in different experimental conditions were processed for flow cytometry, western blotting and *in vitro* NOS specific activity analyses. *Results.* HEMA increases apoptosis percentage, when DMEM containing Phenol red (Phr+) is used, in comparison to Phr-DMEM. Both ROS production and PKCα expression and activation are also increased in Phr+DMEM 96h after HEMA exposure, when compared to Phr-DMEM. The increased specific activity of iNOS, inflammatory enzyme, accompanied by Bax high expression in HGF in response to 96h HEMA, mainly in Phr+ medium, suggest the occurrence of an inflammatory and apoptotic response to such agent. *Conclusions.* These results let us hypothesize that estrogen can amplify the inflammatory response disclosed by gingival fibroblasts to HEMA released monomers.

References

1. Moharamzadeh K et al. Dent Mater 2007:23;40-4.
2. Barnett ME et al. Cell Signal 2007;19:1820-9.

HISTOCHEMICAL AND STEREOLOGICAL CHANGES FOLLOWING CRUSH INJURY OF BRACHIAL PLEXUS TERMINAL BRANCHES IN ADULT RATS

S. Geuna, M. Fornaro, S. Raimondo, L. Muratori, G. Ronchi, S. Bompasso, M.G. Giacobini-Robecchi

Neuroscience Istitute Cavalieri Ottolenghi & Dept. of Clinical and Biological Sciences University of Turin, Italy.
E-mail: stefano.geuna@unito.it

The histochemical and stereological changes occurring after a nerve crush lesion applied to the median, ulnar and radial nerves, both downstream and upstream to the lesion site, were investigated in adult rats. Animals were sacrificed at different time points after the injury and the nerves and the corresponding C5-T1 DRGs were extracted. Distal to the crush lesion, morphological analysis showed that axonal regeneration and maturation was very fast. Regenerated nerve fibers were, at month-1 post lesion, significantly more numerous and densely packed while they were smaller and with a thinner myelin sheath compared to controls. However, after 6 months, values were back to control. Proximal to the crush lesion, morphological analysis of DRGS showed an unusual number of small size cells different from the glial satellite cells. Neurogenesis in the DRGs was then investigated by injecting rats with bromodeoxyuridine (BrDU). Most of the BrDU positive cells belong to the glial family although, some BrDU colocalized with neuronal markers (nestin, Sox-2) suggesting that neurogenesis occurs in adult DRG neurons that undergo peripheral nerve injury. Stereological analysis showed a significant increase in the number of sensory neurons 1 month after nerve-crush injury compared to controls, while at month-6 the number was back to control values. All together our data support the idea that a transitory increase in the number of DRG neurons occur as a consequence of the nerve damage. Evidence of morphological changes in the population of cells surrounding neurons and the immunopositivity for neuronal progenitor markers, suggested the hypothesis that the increased number of neurons is due to undifferentiated precursors localized within the adult DRG.

IMMUNOCYTOCHEMICAL, WESTERN BLOT AND ELISA ANALYSIS EVALUATION OF ASTROGLIAL BIOMARKERS IN ASTROCYTE CULTURES TREATED WITH ALPHA-LIPOIC ACID

V. Bramanti,[1] D. Bronzi,[1] S. Grasso,[1] D. Tomassoni,[2] M. Currò,[3] R. Ientile,[3] F. Amenta,[4] R. Avola[1]

[1]Dept. of Chemical Sciences, Sec. Biochemistry and Molecular biology, University of Catania; [2]School of Bioscience and Biotecnology, University of Camerino; [3]Dept. of Biochemical, Physiological and Nutritional Sciences, University of Messina; [4]School of Pharmacy, University of Camerino, Italy. E-mail: ravola@unict.it

Alpha-lipoic acid (ALA) plays a crucial role as antioxidant and metabolic component of some enzymatic complexes involved in glucose metabolism of different cell types. In the present study, we evaluated the expression of some proliferation and differentiation markers in 15 DIV astrocyte cultures pretreated or not with 0.5 mM glutamate for 24 h and than maintained under chronic or acute treatment with 50 μM R(+)enantiomer or raceme-(ALA). GFAP expression significantly increased after (R+)enantiomer acute-treatment and also in glutamate-pretreated ones. R(+)enantiomer acute-treatment increased vimentin expression, but it decreased after raceme acute-treatment. Nestin expression drastically increased after acute raceme-treatment in glutamate-pretreated or unpretreated cultures, but significantly decreased after (R+)enantiomer acute and chronic-treatments. Cyclin D1 expression much increased in raceme acute-treated astrocyte cultures pretreated with glutamate. MAP-kinase expression slightly increased after (R+)enantiomer acute treatment in glutamate-pretreated or unpretreated ones. Immunocytochemical analysis is well correlated with Western blot and ELISA data. Our results indicate a significant increase of GFAP expression as well as an "up and down" modulation of nestin and vimentin expression in 15 DIV astrocyte cultures after chronic or acute treatment with raceme or (R+)enantiomer ALA. These preliminary findings may better clarify antioxidant and metabolic role played by ALA in proliferating and differentiating astrocyte cultures suggesting an interactive cross-talk between glial and neuronal cells, after brain lesions or damages.

THE CHOLINERGIC SYSTEM OF TWO INBRED MOUSE STRAINS (DBA/2J AND C57/BL6J), DISPLAYING DIFFERENT BEHAVIORS AND MEMORY TASKS: IMMUNOHISTOCHEMICAL AND HISTOCHEMICAL STUDY

G. Vivacqua,[1] R. Vaccaro,[1] A. Casini,[1,2] H. Kimura,[3] L. D'Este[1,2]

[1]Dept. of Anatomic, Histologic, Forensic and Locomotor Apparatus Sciences, Sapienza University of Rome, Rome, Italy; [2]Research Center "Daniel Bovet", Sapienza University of Rome, Rome, Italy; [3]Molecular Neuroscience Research Center, Shiga University of Medical Science, Otsu, Japan.

Mice of the inbred lines C57BL/6J and DBA/2J show strain dependent behavioral differences, which have been correlated with variations in the organization of brain neuronal systems, including cholinergic system. A comparative neuromorphological study has been performed, using choline acetyltransferase (ChAT) immunohistochemistry, acetylcholine esterase staining and immunohistochemistry for specific neuropeptides involved in cholinergic transmission. In different forebrain regions, C57 mice are characterized by a lower ChAT immunoreactivity compared to DBA mice. Unlikely, the acetylcholine turnover seems drastically increased in the dentate gyrus of DBA mice, compared to C57 mice. Galaninergic projections are predominant in DBA mice, whereas, neurotensin seems highly expressed in C57 mice. Moreover, in DBA mice, the huge cholinergic innervation from the septal nuclei, belongs to a reduction of calretinin immunoreactive mossy cells in the dentate gyrus. Our study provides neuroanatomical basis underlying the different memory performances of the two mouse stains and discloses new avenues to understand cholinergic mechanisms in neurological diseases.

References

Kenney and Gould. Mol Neurobiol 2008;38:101-21.
D'Este et al. J Chem Neuroant 2007;33;67-74.
Hasselmo. Curr Opin Neurobiol 2006;16:710-5.
Passino et al. Hippocampus 2002;12:63-75.
Schurmans et al. Proc Natl Acad Sci USA 1997;94:10415-20.

NEURAL SYSTEM REARRANGEMENT DURING THE METAMORPHOSIS OF THE HYDROID *CLAVA MULTICORNIS*

R. Pennati,[1] A. Dell'Anna,[1] P. Pagliara,[2] G. Scarì,[1] G. Zega,[1] S. Piraino,[2] F. De Bernardi[1]

[1]Dept. of Biology, University of Milano, and [2]Dept. of Sciences and Environmental Biological Technologies, University of Salento, Italy.
E-mail: fiorenza.debernardi@unimi.it

The planula larva of the hydroid *Clava multicornis* has a complex nervous system. It is characterized by the presence of distinct populations of neural elements concentrated at the anterior end representing the sensory pole of this life stage. The neurons can be identified by the presence of different neuropeptides: GLWamide positive cells are arranged in a dome-like organization in the anterior most region and are followed by a belt of RFamide positive ones, both types being connected to a basiectodermal anterior plexus.[1] During the metamorphosis, the larva attaches to the substrate with the anterior end that develops into the basal foot region of the polyp, while the posterior end forms the oral region. In the polyp of the hydrozoan *Hydractinia echinata* GLWamide and RFamide positive neurons are concentrated around the mouth and in the tentacles, at the opposite side respect to the position occupied in the larva.[2] To elucidate the fate of the neural cells in *C. multicornis*, we investigated the distribution pattern of GLWamide and RFamide positive sensory cells at different stages of metamorphosis. We observed that immunoreactivity is still present at the anterior end of the larva during early settlement, but gradually disappeared through the following stages of metamorphosis. Only in late stages positive cells appeared around the mouth of the newly formed polyp. By TUNEL assay, apoptotic nuclei were identified in the anterior end of the settled larva, in the same region occupied by sensory cells. These results suggest that at least part of the neurons of the larva degenerates during metamorphosis by apoptosis and that at least part of the adult nervous system is formed by *de novo* differentiation. Understanding how the nervous system is rearranged during the metamorphosis of basal metazoans can help to elucidate mechanisms of neural plasticity in higher metazoans.

References

1. Piraino S. et al., J. Comp. Neurol. 2011; DOI 10.1002/cne.22614.
2. Seipp S. et al., Invert. Neurosci. 2010;10:77-91.

EFFECTS OF EXPOSURE TO NATURAL AND SYNTHETIC ASBESTOS TREMOLITE: AN *IN VITRO* MODEL FOR POTENTIAL TOXICITY

A. Pugnaloni,[1] G. Lucarini,[1] F. Giantomassi,[1] A. Bloise,[2] E. Belluso[3,4]

[1]Dept. of Molecular Pathology and Innovative Therapies, Patologia Molecolare e Terapie Innovative, Istologia, University Politecnica Marche, Ancona; [2]Dept. of Earth Sciences, University of Calabria, Cosenza; [3]Dept. di Mineralogical and Petrological Sciences, University of Turin; [4]Institute of Geosciences and Earth Resources CNR-Unity of Turin, Turin, Italy. E-mail:armanda.pugnaloni@univpm.it

Asbestos is the name of naturally inorganic mineral silicates whose pathogenetic mechanisms are not entirely understood. The effects of two different samples of asbestos tremolite, Natural Asbestos Tremolite (NAT) from Brachiello, Val d'Ala, Piedmont Region, and Synthetic Asbestos Tremolite (SAT),[1] both previously mineralogically characterized, were *in vitro* tested in alveolar epithelial A549 cells representing the first target of inhaled micro-environmental contaminants. Functional and structural damages were evidenced by viability, motility and morphological investigations. Phalloidin labelling showed irregular distribution of cytoskeletal actin; immunohistochemical investigations evidenced abnormal expression of VEGF,[2] Cdc42[3] and β-catenin, considered risks indicators for cancer development.[4] NAT effects resulted in survival of compromised cells highly expressing VEGF while SAT fibres exerted a direct cytotoxic effect and can be considered as standard sample in several investigations. The potential health hazard of NAT fibres *in vivo*, is related to their iron content and ROS generation capacity providing the opportunity to elucidate the effect of asbestos on cancer induction.

References

1. Bloise A et al. Eur J Mineral 2008;20:1027-33.
2. Bremnes R et al. Lung Cancer 2006;51:143-58.
3. Price L and Collard J Semin Cancer Biol 2001;11:167-73.
4. Pugnaloni A. et al. Acta Histochem 2010;112:133-46.

POSTER SESSION

STRUCTURAL AND FUNCTIONAL FEATURES OF SKELETAL MUSCLE CELL NUCLEI ARE MODULATED BY PHYSICAL EXERCISE IN OLD MICE

M. Malatesta,[1] P. Fattoretti,[2] M. Giagnacovo,[3] C. Pellicciari,[3] C. Zancanaro[1]

[1]Dept. of Neurological, Neuropsychological, Morphological and Motor Sciences, Anatomy and Histology Section, University of Verona; [2]Cellular Bioenergetics Lab., INRCA, Ancona; [3]Dept. of Animal Biology, Lab. of Cell Biology, University of Pavia; Italy.
E-mail: manuela.malatesta@univr.it

Ageing is associated with a progressive loss of muscle mass, strength and function, a condition known as sarcopenia which represents an important risk factor for physical disability in elderly. The mechanisms leading to sarcopenia are still largely unknown and no specific therapy is presently available to counteract its onset or progress. Many studies have stressed the importance of physical exercise as an effective approach to prevent/limit the age-related muscle mass loss. In this work, the effects of physical training on pre-mRNA pathways have been investigated in quadriceps and gastrocnemius muscles of old mice by ultrastructural cytochemistry: structural and *in situ* molecular features of myonuclei and satellite cell nuclei have been compared in exercised *versus* sedentary old mice, using adult individuals as control. Our results demonstrated that in myonuclei of old mice physical exercise stimulates pre-mRNA transcription, splicing, and export to the cytoplasm, likely increasing muscle protein turnover. In satellite cells the effect of physical exercise seems to be limited to the reactivation of some factors involved in the transcription-splicing apparatus without increasing RNA production, probably making these quiescent cells more responsive to activating stimuli. Our study suggests that the stimulation of skeletal muscle nuclei by physical exercise may induce increased protein synthesis in muscle fibres of old animals and satellite cell activation, thus limiting the age-related muscle wasting.

ALTERATION OF RNA PROCESSING IN SKELETAL MUSCLE NUCLEI OF PATIENTS AFFECTED BY MYOTONIC DYSTROPHY

M. Malatesta,[1] M. Giagnacovo,[2] R. Cardani,[3,4] G. Meola,[5] C. Pellicciari[2]

[1]Dept. of Neurological, Neuropsychological, Morphological and Motor Sciences, Anatomy and Histology Section, University of Verona; [2]Dept. of Animal Biology, Lab. of Cell Biology, University of Pavia; [3]Dept. of Molecular Biology and Biotechnologies, University of Milan; [4]Centre for the Study of Neuromuscular Diseases - CMM; [5]Dept. of Neurology, IRCCS Policlinico San Donato, University of Milan; Italy. E-mail: manuela.malatesta@univr.it

Myotonic dystrophies type 1 and 2 (DM1 and DM2) have highly variable clinical phenotypes including muscle weakness and atrophy, and several extramuscular manifestations. In either forms, the presence of expanded nucleotide sequences causes the accumulation of mutant transcripts in the nucleus: this would deregulate the function of some RNA-binding proteins and hamper mRNA processing. However, there is no direct evidence that structural changes do actually occur in the organization of the cell nuclear domains in skeletal muscle of DM patients. We therefore performed a study by immunoelectron microscopy on *biceps brachii* biopsies from DM1, DM2 and healthy subjects, and provided the first ultrastructural evidence on the relocation of some nuclear ribonucleoprotein-containing structures and molecular factors involved in pre-mRNA transcription and maturation in dystrophic myonuclei. Our results demonstrated an accumulation of splicing and cleavage factors in myonuclei of DM1 and DM2 patients, suggesting an impairment of post-transcriptional pre-mRNA pathways. The accumulation of the expanded sequences in DM myonuclei would therefore impair functionality of the whole splicing machinery, and slow down the intranuclear molecular trafficking; as a consequence, the capability of myonuclei to respond to anabolic stimuli would be reduced, thus contributing to muscle wasting.

PGF2α ACTIVATES DNA DAMAGE CHECK-POINT MOLECULES ON OSTEOBLASTS

D. Agas, M.G. Sabbieti, L. Marchetti

School of Bioscience and Biotechnology, University of Camerino, Italy. E-mail: luigi.marchetti@unicam.it

Chronic inflammation and neoplastic transformation are associated since it is well known that an increased cancer risk exists in chronically inflamed tissues. Cells, continuously encountering DNA damage and failuring to properly DNA repair, can lead to various disorders, including enhanced rates of tumor development. DNA damage checkpoint molecules (ATR, Chk1, H2AX) inhibit the cycline-dependent kinase machinery, which is known to coordinate DNA replication and chromosome partition.[1] We previously demonstrated that the inflammatory mediator prostaglandin F2α (PGF2α) up-regulated multiple proliferative and survival signals on osteoblasts[2] and enhanced nuclear accumulation and co-localization of Bcl2 and c-Myc oncoproteins.[3] Since the nuclear localization of Bcl2 is associated with mutagenesis and genome instability, the goal of this study was to examine whether PGF2α could participate on DNA damage. Indeed, primary calvarial osteoblasts showed a statistically significant increase of base lesion/cell after 24 h of PGF2α treatment. In addition, western blotting data evidenced that PGF2α increased phospho-ATR which, in turn, activated Chk1 kinase. Interestingly, immunofluorescence and western blotting data also demonstrated an increased level of phospho-H2AX which is known to be activated in the chromatin microenvironment surrounding a DNA double-strand break. On this regard, it is also important to note that PGF2α induced upregulation of the above check-point molecules and, in parallel, stimulated cell cycle progression. Further studies are in progress to better clarify the biological relevance of these findings.

References

1 Zhou B.B. et al., Nature. 2000, 408:433-9.
2. Agas D. et al., J Cell Physiol 241: 145-52.
3. Sabbieti M.G. et al., J Cell Physiol 2010, 224:465-74.

ULTRASTRUCTURAL IMMUNOLOCALIZATION OF THE SENESCENCE-RELATED PROTEIN TERMININ IN HUMAN FIBROBLASTS

M. Giagnacovo,[1] M. Malatesta,[2] P. Veneroni,[1] E. Wang,[3] C. Pellicciari[1]

[1]Dept. Animal Biology, Lab. Cell Biology, Univ. Pavia; [2]Dept. Neurological, Neuropsychological, Morphological and Motor Sciences, Anatomy and Histology Section, Univ. Verona, Italy; [3]Dept. Biochemistry and Molecular Biology & Gheens Center on Aging, Univ. Louisville, USA. E-mail: pelli@unipv.it

Terminin is a cytoplasmic protein originally found in human fibroblasts, where three forms of different molecular weight

were found: the 90 kDa form, in young fibroblasts; its cleaved 60 kDa fragment, in irreversibly growth-arrested senescent cells; a 30 kDa fragment, in apoptotic cells. All the forms can be detected by the monoclonal antibody 1.2 in Western blots, whereas in immunohistochemistry the same antibody only recognizes the insoluble 60 and 30 kDa forms. Although the role of terminin and its cleavage products is still unknown, the immunopositivity for this protein is considered as a marker for cell senescence/ageing, terminal differentiation, and apoptosis. Here, the intracellular distribution of terminin was investigated, using a combined immunohistochemical approach at light and transmission electron microscopy (TEM). As a model system, asynchronous primary cultures of normal human fibroblasts at different passages were selected. For light microscopy, indirect immunodetection methods were used, utilizing either fluorochrome-labelled or horseradish peroxidase (HRP)-conjugated secondary antibodies finally revealed by diaminobenzidine (DAB); for TEM, we used either pre-embedding immunodetection with HRP-DAB or post-embedding gold-immunolabelling. All methods consistently identified cytoplasmic vacuoles as the main site of accumulation of immunodetectable terminin; based on their ultrastructural morphology, these membrane-bounded organelles may be likely identified as phagolysosomes or residual bodies.

ULTRASTRUCTURAL FEATURES OF MYOTUBES DERIVED FROM MYOBLASTS OF PATIENTS AFFECTED BY MYOTONIC DYSTROPHY TYPE 2, AFTER SENESCENCE *IN VITRO*

M. Giagnacovo,[1] M. Costanzo,[1] R. Cardani,[2,3] P. Veneroni,[1] C. Pellicciari,[1] G. Meola[4]

[1]Dept. of Animal Biology, Lab. of Cell Biology, Univ. Pavia; [2]Dept. of Molecular Biology and Biotechnologies, Univ. Milan; [3]Centre for Study of Neuromuscular Diseases - CMM; and [4]Dept. of Neurology, IRCCS Policlinico San Donato, Univ. Milan, Italy. E-mail: pelli@unipv.it

Myotonic dystrophy type 2 (DM2) is an autosomal dominantly-inherited disease with multi-systemic clinical traits. It is caused by the expansion of a CCTG tetranucleotide repeat in the first intron of the zinc finger protein 9 gene, in 3q21. The expanded-CCUG-containing transcripts are retained in cell nuclear domains (foci) where several splicing factors are sequestered; the resulting general alteration of the pre-mRNA post-transcriptional pathway is likely responsible for the multifactorial phenotype of DM2 patients. It has been noted that in DM2 patients skeletal muscle regeneration is decreased, suggesting an impaired responsiveness of satellite cells to regeneration stimuli, as much as it occurs in aging muscles. In search of a still lacking mechanistic explanation for muscle weakness and atrophy of DM2 patients, we investigated the differentiation of senescing DM2 myoblasts, and the development of the resulting myotubes: namely, some structural characteristics of DM2 myoblasts and myotubes grown in culture for increasing times have been studied by fluorescence and transmission electron microscopy. Several features (i.e., shorter myotubes with a relatively low number of nuclei, progressive cytoskeletal disorganization, partial mitochondrial degeneration) have been observed to occur in the myotubes derived from myoblasts at higher passages. This strongly argues in favour of the involvement of satellite cell senescence in the reduced regenerative potential of dystrophic muscles.

FOLLICLE-STIMULATING HORMONE INDUCES CHOLANGIOCYTES GROWTH IN COURSE OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

R. Mancinelli,[1] A. Franchitto,[1] P. Onori,[2] G. Carpino,[3] S. Brozzetti,[4] L. Izzo,[4] A. Bolognese,[4] D.M. Jefferson,[5] S. Glaser,[6] G. Alpini,[6] E. Gaudio[1]

Depts of [1]Anatomical, Histological, Forensic medicine and Orthopedic Sciences, [4] Surgery "P. Valdoni", University of Rome "Sapienza", Italy; [2]Dept of Experimental Medicine, University of L'Aquila, Italy,[3]Dept of Health Science, University of Rome "Foro Italico", Italy; [5]Tufts University, Boston, USA; [6]Scott & White Digestive Disease Research Center, Texas A&M Health Science Center, USA.

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder characterized by the progressive development of renal and hepatic cysts that bud from biliary epithelium.[1-2] Follicle-Stimulating Hormone (FSH) has been demonstrated to be a trophic factor for the biliary cells in normal and experimental model of bile duct ligation rat (BDL).[3] On this basis, the role of FSH has been investigated *in vivo* on cholangiocytes from cysts of patients with ADPKD and *in vitro* on normal human cholangiocytes (H69) and on an immortalized cell line obtained from the epithelium lining the hepatic cysts from patients with ADPKD (LCDE). By immunohistochemistry and immunofluorescence, we found that cyst epithelium: (i) expresses FSHR and FSH, (ii) proliferates in relation with FSH higher presence, and (iii) colocalizes pERK and c-myc with proliferation markers. These results indicate that FSH has an important function in the regulation of cystic growth via cAMP/ERK mechanism, confirming that this pathway provides a target for medical therapy of hepatic cysts during ADPKD.

References

1. Onori P. et al. Dig Liver Dis. 2010;42:261-71.
2. Alvaro D. et al. Am J Pathol 2008;172:321-32.
3. Mancinelli R. et al. Am J Physiol GL 2009;297:G11-26.

ACE I AND ACE II: IMMUNOHISTOCHEMICAL STUDY IN PRIMARY PTERYGIUM AND PRIMARY CULTURES FROM PTERYGIUM

P. Demurtas,[1] M.T. Perra,[1] C. Maxia,[1] I. Zucca,[2] M. Fossarello,[2] S. Lai,[1] F. Piras,[1] P. Sirigu[1]

[1]Department of Cytomorphology, [2]Department of Surgical Sciences, Eye Clinic, University of Cagliari, Italy. E-mail: cmaxia@unica.it

Pterygium is a disease of the ocular surface associated with chronic UV exposure and characterized by proliferation, inflammatory infiltrates, fibrosis, and angiogenesis. Recent report assumes Renin-angiotensin system (RAS) as a new potential mediator for growth factor and an immunomodulator that influences cell proliferation, apoptosis, tissue fibrosis and is involved in inflammatory processes. Classically, RAS is a coordinated hormonal cascade that acts in cardiovascular, renal, and adrenal functions mainly by the action of Angiotensin II (Ang II), but as inflammatory mediator, Ang II enhances vascular permeability through prostaglandins and vascular endothelial growth factor, and contributes to the recruitment of inflammatory cells by inducing chemokines and adhesion molecules. Furthermore, Ang II may act in inflammatory responses in a non-hemodynamic manner. Ang II is generated by enzymatic action of Angiotensin Converting Enzyme (ACE I). Besides the action of Ang II, exists an "alternative" way mediated by Angiotensin-(1-7) [Ang-(1-7)]. Ang-(1-7) is an alternative metabolite of the RAS system with actions in opposition to

those of Ang II. It is generated by enzymatic action of Angiotensin Converting Enzyme II (ACE II). The aim of present study has been to investigate, by immunohistochemistry, on paraffin-embedded primary pterygium sections and primary cultures from human pterygium cells, the possible alteration of ACE I and ACE II compared with normal conjunctiva. Our preliminary results may indicate a possible downregulation of ACE I in pterygium cell cultures compared with normal conjunctiva.

PATTERN OF EXPRESSION OF WOLFRAMIN IN NORMAL AND PATHOLOGICAL HUMAN ENDOMETRIUM

A. Lucariello,[1] L. Manente,[1] O. Boccia,[2] A. Perna,[1] C. Sellitto,[1] R. Palladino,[3] L. Cobellis,[2] A. De Luca[1]

[1]Dept. of Medicine and Public Health, Section of Human, Clinic and Radiology Anatomy, Second University of Naples; [2]Dept. of Gynaecology, Obstetric and Reproductive Science, Second University of Studies of Naples; [3] Luigi Curto Hospital, Polla – SA; [4] Dept. of Biological Sciences, Section of Evolutionary and Comparative Biology, University of Naples Federico II; Italy. E-mail: antonio.deluca@unina2.it

Wolframin is a transmembrane glycoprotein of endoplasmic reticulum consisting of 890 amino acids. It is encoded by the *WFS1* gene, mutated in the Wolfram syndrome, also called DIDMOAD, an autosomal recessive disorder defined by the association of diabetes mellitus, optic atrophy, and further organ abnormalities. However the physiological function of this protein remains totally unknown. To gain further insight into the pathogenesis of diseases associated with *WFS1* mutations, we conducted a study to investigate its pattern of expression in physiologic and pathologic human endometrium by immunohistochemistry. For this purpose, 60 samples of physiologic endometrium and 60 samples of pathologic endometrium were used. In physiologic endometrium, we observed a light increase of wolframin from proliferative to secretory phase where wolframin was localized in the glands, stroma and cells lining blood vessels. In menopause, wolframin expression increased with a glandular and stromal localization. In pathologic endometrium, we observed an increase of wolframin expression from hyperplasia to polyps until a higher expression in carcinoma tissues. In human endometrium, wolframin seems to have a role in differentiation program. Deregulation of these functions may induce the onset of several endometrial pathologies.

IMMUNOCYTOCHEMICAL DETECTION OF rMnSOD AND APOPTOSIS IN HIGH-RISK PEDIATRIC LEUKEMIA CELLS

A. Di Santi,[1] F. Basile,[1] V. D'Angelo,[3] A. Iannotta,[3] M. Ramaglia,[3] A. Borrelli,[2] A. Schiattarella,[2] P. Indolfi,[3] F. Casale,[3] A. Mancini,[2] A. Pica[1]

[1]Biol. Sci., University Federico II, Naples, Italy; [2]Mol. Biol. & Viral Oncogen., Nat. Cancer Inst. Pascale, Naples, Italy; [3]Pediatric Oncology, Second University, Naples, Italy. E-mail: alessandra.pica@unina.it

High-risk (HR) pediatric acute lymphoid leukemias (ALL) B and T cells have poor prognosis: a complete remission can be achieved with complex protocols.[1] In this study, the rMnSOD action was tested on pediatric B and T high-risk ALL cells. LSA-rMnSOD, the recombinant isoform of SOD secreted by liposarcoma, exerts an oncosuppressive action.[2] The internalization of rMnSOD at scalar concentrations (20-0.2 μg/mL) in leukemic cells was demonstrated by LM and TEM immunocytochemistry. Proliferation tests was studied by TB and MTT assay. The intense immunolabeling after 20 μg/mL rMnSOD-treatment decreased as concentrations were reduced. Early signs of apoptosis were detectable with a major modulation of proapoptotic Bax and a negative modulation of anti apoptotic Bcl-2. Conversely, we observed in ALL B-HR a down phosphorylation of AKT and ERK respect to diagnosis, with a decrease in proliferation (MTT= 27%). In ALL T-HR none significative variation was observed after treatment. Thus, rMnSOD enters in leukemic cells, suppressing, by high concentrations of H_2O_2, their proliferation.[3] Biomolecular detection of apopototic proteins showed that the 2 μg/mL was most effective dose of rMnSOD able to trigger apoptosis of leukemic cells. rMnSOD could become an innovative anti-cancer drug for treatment of high-risk leukemia in emerging target therapies because of its action in pro survival signaling.

References

1. Graux C. Transfus Apher Sci. 2011, 44, 183–9.
2. Mancini A. et al. Int. J. Cancer 2008, 123: 2684-95.
3. Battisti V. et al. Clin Biochem.2008; 41:511-8.

IMMUNOLOCALIZATION OF NITRIC OXIDE SYNTHASE ISOFORMS IN THE OLFACTORY EPITHELIUM OF THE *AMBYSTOMA TIGRINUM*

P. LoCascio,[1] D. Palombieri,[1] D. Zaccone,[3] R. Zena,[1] M. Calò,[2] C. Calabrò[3]

[1]Dept. of Food and Environmental Science,[2]Dept. of Experimental Sciences and Applied Biotechnology, [3]Dept. of Animal Biology and Marine Ecology, University of Messina, Italy. E-mail: deborahpalombieri@alice.it

Nitric oxide (NO) is a molecule identified both as a neuromodulator and as an intracellular messenger, produced from L-arginine by nitric oxide synthase (NOS). Since NO is involved in olfactory perception both invertebrates[1] and vertebrates[2], the expression of nNOS and eNOS, isoforms of NOS, was examined in the olfactory epithelium (OE) of *Ambystoma tigrinum*. OE[3], lines the nasal cavity and vomeronasal organ of *A. tigrinum*, is formed by three characteristic cellular types: sustentacular cells, basal cells and olfactory receptor neurons (ORN). The ORN consist of an elongated cell body, a long dendrite and an axon emerging from the inferior region of the cell body and entering the olfactory nerve. The dendrite is directed apically toward the epithelium surface and ends an apical knob with cilia or microvilli, forming the mucociliary/microvillar complex. Sustentacular cells have been associated with the regulation of the mucus layer composition. Basal cells presumably give rise to new ORN. In the OE of nasal cavity Bowman's glands was founded nNOS immunoreactivity, mainly localized at the ORN dendrites, the mucociliary/microvillar complexes, Bowman's glands secretory and duct cells. eNOS immunoreactivity was localized at the lamina propria of arterioles. Thus, present results suggest that NO might be involved in several functions of the OE: olfactory signal transduction, blood flow regulation, modulation of Bowman's glands secretion, ORN development.

References

1. Gelperin A. et al. J Neurophys 2000;83:116-27.
2. Wenisch S. et al. J Histochem 2000;32:381-8.
3. Woodley SK. J CompPhysiol 2010;196:713-27.

STUDY ON FERTILIZED EGGS IN THE EGG-CAPSULES OF *JANTHINA PALLIDA* (THOPSON, 1840) MOLL. GAST. PROS.

C.Calabrò,[1] S.Giacobbe,[1] C.Bertuccio,[2] A. Alesci,[3]
D. Palombieri,[3] P. LoCascio[3]

[1]Dept. of Animal Biology and Marine Ecology, [2]Dept. Life Science, [3]Dept. of Food and Environmental Science, University of Messina, Italy.
E-mail: deborahpalombieri@alice.it

The genus Janthina, includes no more than ten species of Mesogastropoda Prosobranchia Mollusca widespread in the tropical and subtropical waters of all oceans;[1] floating on the water-surface by means of peculiar rafts made of air bubbles. The specific adaptations of Janthinidae to pelagic life have been of interest to several authors.[2,3] Still reproductive biology is not clear, therefore in this regard investigations carried out the different stages of development of fertilized eggs of *Janthina pallida* individuals, collected on the east shore of Strait of Messina on weather conditions that allowed the stranding. In large specimens a hermaphrodite gonad, with an indistinct separation between male and female zones with germinative cells in different stages of spermatogenesis and ovogenesis, was observed. Histochemical and immunohistochemical analysis revealed respectively in yolk globules, proteins, glycoproteins, proteoglycans and glycoconiugates by conferring to α-D-glucose, α-D-mannose, α-L-fucose and β-D-galactose sugar radicals and vitellogenin. The simultaneous presence in the adults of germinal cells, in all stages of maturity and of embrionate eggs in the egg-capsules, suggests, in this species, a possible self-fertilizzation ability in positive environment.

References

1. Fretter and Graham, The Ray Society 1962;161:1-755.
2. Ankel W.M. Cell and Tissue Res 1930;11.491-608.
3. Calabrò C.et al. 2011, International Journal of Malacology, in press.

SARCOGLYCANS IN RAT'S CEREBRAL AND CEREBELLAR CORTEX: AN IMMUNOHISTOCHEMICAL STUDY

G. Anastasi, F. Trimarchi, D. Di Mauro, G. Rizzo, G. Vermiglio, L. Magaudda

Dept. of Biomorphology and Biotechnology, University of Messina, Italy. E-mail: ddimauro@unime.it

The sarcoglycan sub-complex (SGC) is a transmembrane protein system which plays a key role in sarcolemma stabilization during muscle activity and in cellular signalling. In cerebral and cerebellar cortex, only ε-sarcoglycan presence it was demonstrated because its involvement in Myoclonus Dystonia syndrome (MDS). On this basis we carried out an indirect immunofluorescence study on normal rat's cerebral and cerebellar cortex where we have tested α-, β-, γ-, δ- and ε-sarcoglycans expression. Our results showed that both in cerebral and cerebellar cortex all of tested sarcoglycans are present, by a "spot-like" fluorescence pattern, with spots of 0.5-2 μ in middle-size laid out mainly around cellular soma of neurons and glial cells. In cerebral cortex, though all of sarcoglycans are present, a staining pattern variability for each sarcoglycan exists. In fact, our data show that the distribution pattern level of some sarcoglycan in anterior sections, corresponding to fronto-parietal region, is different to their distribution pattern level in posterior sections, corresponding to parieto-occipital region. In cerebellar cortex we found that in neurons sarcoglycans positivity is localized only in single layer present in a middle region of the section. These results, showing a distribution of SGs around cellular soma of neurons and glial cells in cerebral and cerebellar cortex, suggest that in brain sarcoglycans may play a key role in cellular signalling, regulating membrane receptors assembly; we can also speculate that the staining pattern variability detected in cerebral cortex could marks a specific receptor network present in each cerebral cortex areas.

INTEGRINS IN MASSETER MUSCLE IN UNILATERAL CROSSBITE PATIENTS: AN IMMUNOHISTOCHEMICAL STUDY

G. Cutroneo, G. Isola, A.Centofanti, M. Runci, E. Magaudda, A. Favaloro

Dept. of Biotechnology and Biomorphology, University of Messina, Italy. E-mail: afavaloro@unime.it

Integrins are heterodimeric cell surface integral membrane proteins that play a key role in cell adhesion, differentiation, remodelling and tissue repair. B1D isoform is synthetized only in skeletal and cardiac muscle, while very low amounts of β1A were detected by immunoblot in striated muscles. B1D isoform was associated with α7A and α7B in adult skeletal muscle. Although many studies have been performed on the integrins in adult skeletal muscle, insufficient data exist on behaviour of these proteins in masseter muscle. About this muscles it was demonstrated that they have several differences in respect to limb and trunk muscles. Generally, fibers in the masseter muscle are smaller than fibers in limb and trunk muscles. Consequently, the smaller muscle fibers may be advantageous for the jaw muscles. On this basis, we performed an immunohistochemical study in order to analyze the behaviour of integrins in normal masseter muscle of both side; moreover, to better comprehend the role of these proteins, we also analyzed masseter muscles of patients affected by unilateral crossbite. Interestingly, our result showed that, on patients affected by right crossbite, the integrins are substantially less, in both masseters, than those observed in control subjects; in right masseter, the amount of integrin appeared less than the amount of integrins detected in left counterpart. Since kinematics and electromyography study have been demonstrated that masseters of the crossbite side were less active than non-affected side, our results, showing a decrease of integrins in the masseter of crossbite side, allow to hypothesize that the integrins, and in particular α7A and β1A integrins, could play a crucial role in the control of contraction activity.

DETECTION OF MMP-2 IN HUMAN DENTIN MATRIX

A. Mazzoni,[1] P. Gobbi,[2] V. Papa,[3] F. Nato,[1] A. Ruggeri Jr,[1] L. Breschi,[4] G. Mazzotti[2]

[1]Dept. SAU&FAL, Univ. of Bologna, Bologna; [2]Dept. STeVA, Univ. of Urbino "Carlo Bo", Urbino; [3]Dept. of Sport, Sciences & Health, Univ. of Cassino, Cassino; [4]Dept. of Biomedicine, Univ. of Trieste, Trieste, Italy
E-mail: pietro.gobbi@uniurb.it

Matrix metalloproteinases (MMPs) are important components in many biological and pathological processes because of their ability to degrade all extracellular matrix (ECM) components. The aim of the study was to identify MMP-2 in human dentin by immunohistochemical and biochemical methods. Dentin cryo-fractured fragments were obtained from human sound teeth, partially decalcified in 0.5 M EDTA pH 7.4 for 30min and submitted to a pre-embedding immunolabeling technique, using primary monoclonal antibodies anti-MMP-8 and exposed to a secondary antibody conjugated with gold nano-particles. Observation was performed by means of a FEI-SEM. The presence of MMP-2 was additionally assayed and quantified using a colorimetric assay system that allows direct

measurement of MMP-2 levels. The immunohistochemical analysis revealed an intricate three-dimensional network of type I collagen and positive immunolabeling patterns for MMP-2 showing its distribution along the collagen fibrils. The colorimetric assay allowed identification and quantification of MMP-2 in human dentin showing higher presence of MMP-2 in mineralized dentin, while it significantly decreased in the partially demineralized dentin The role and function of dentin MMPs is not well known, but they have shown to contribute to auto-degenerative processes in dentin, such as inflammation of dental pulp, progression of caries lesions. This study demonstrated using an immunohistochemical and a biochemical approach that MMP-2 is an intrinsic component of the human dentin organic matrix, with probable roles in dentin matrix turnover and degenerative processes.

MATRIX METALLOPROTEINASES AND TISSUE INHIBITORS OF METALLOPROTEINASES IN HUMAN SOUND DENTIN

F. Nato,[1] A. Mazzoni,[2] A. Maklaj,[1] A. Ruggeri,[2] P. Gobbi,[1] L. Breschi,[3] G. Mazzotti[2]

[1]Univ. of Urbino "Carlo Bo", [2] Univ. of Bologna, [3]Univ. of Trieste, Italy. E-mail: pietro.gobbi@uniurb.it

Matrix metalloproteases (MMPs) are enzymes calcium/zinc-dependent proteinases and operate a specific proteolytic activity on most constituents of the extracellular matrix.[1] The effects of MMPs are regulated by tissue inhibitors of metalloproteinases (TIMPs),[2] small regulatory proteins that can bind ubiquitously different enzyme forms. Different studies showed that MMPs are also involved in dentinogenesis as well as in autodegenerative processes (MMP-2 and MMP-9), such as the degradation of dentin matrix in caries lesions.[3] The purpose of this study was to identify different enzyme isoforms in association with TIMPs, through co-immunoprecipitation/immunoblotting and the evaluation of enzyme activity by zymography. Proteins were extracted from human dentin powder previously demineralized with 1% H_3PO_4 for 10 min. Extraction buffer is composed of 5 mM $CaCl_2$, 100 mM NaCl, 0.1% Triton X-100, 0.1% NONIDET, 0.1 mM $ZnCl_2$, 0.02% NaN_3 and protease inhibitors in 50 mM Tris-HCl pH 6. The proteins extracted were divided for zymography analysis and for co-immunoprecipitation and subsequent immunoblotting against MMPs and TIMPs. Zymography analysis of extracted enzymes showed the presence, in dentin, of both MMP-2 and MMP-9. Co-immunoprecipitation/immunoblotting analysis show the association TIMP1/MMP-2 and TIMP1/MMP-9. Within the human sound dentin, different forms of MMPs in association with TIMP-1 can be detected. Their association is finely adjusted and an imbalance could lead to caries pathology and failure of adhesive systems.[4]

References

1. Visse R, Nagase H. Circ Res. 2003;92:827-39.
2. Gomez DE et al. Eur J Cell Biol 1997;74:111-22.
3. Tjäderhane L et al. J Dent Res 1998;77:1622-9.
4. Breschi L et al. Dental Mater 2008;24:90-101.

INTERACTION OF BIOPHYSIC STIMULI ON CHONDROGENIC DIFFERENTIATION OF MSCs: PRELIMINARY RESULTS ON THE EVALUATION OF ANTI-INFLAMMATORY EFFECT OF ELECTRO-MAGNETIC FIELDS

C. Palumbo,[1] M. Ferretti,[1] L. Bertoni,[1] F. Cavani,[1] M. Benincasa,[1] A.P. Taronna,[1] P. Sena,[1] S. Setti,[2] R. Cadossi[2]

[1]Dept. of Biomedical Sciences, Human Morphology Section, University of Modena-Reggio Emilia; [2]IGEA S.p.A. Carpi, Modena, Italy. E-mail: carla.palumbo@unimore.it

The aim of the present study is to investigate *in vitro* chondrocyte-like cells treated with electromagnetic fields to evaluate over time maintenance of chondrocyte phenotype, in presence of pro-inflammatory cytokines (IL-1β). Mesenchimal stem cells taken from bone marrow were cultured (in pellet) in medium conditioning towards the chondrogenic lineage. The targets are firstly to standardize the method to obtain chondrocyte pellets in terms of a) type/amount of withdrawal, b) time/degree of differentiation, and c) amount of extracellular matrix production; secondly, to extend over time chondrocyte differentiation, checking the phenotype maintenance, after adding pro-inflammatory cytokines in culture medium with/without the application of electromagnetic fields (device provided by IGEA-Carpi). The pellets obtained were coltured for different times (21, 28, 34 days), verifying the presence of type 2 collagen (index of chondrocyte differentiation). The best differentiation was obtained after 28 days of culture. In such pellets, after inflammatory induction and application of electromagnetic field (1.7mT, 75Hz) for 15 days, the observations showed that after about 12 days of treatment the amount of PGE2 in medium decreases (31%) while the proteoglycan production slightly increases (2%). In conclusion, electromagnetic fields could be proposed (if the results will be confirmed) in preventing chondrocyte de-differentiation due to inflammation induced by IL-1β, to integrate regenerative medicine techniques in the healing of cartilage lesions.

CARDIAC TISSUE REGENERATION BY EXTRACORPOREAL SHOCK WAVE THERAPY

V. Romano,[1] R. Miraglia,[1] C. Castaldo,[1] D. Nurzynska,[1] F. Di Meglio,[1] A. De Angelis,[2] E. Piegari,[2] S. Montagnani[1]

[1]Dept. Biomorphological and Functional Sciences and [2]Dept. Experimental Medicine, Second University of Naples; Naples, Italy. E-mail: montagna@unina.it

Recent studies indicate that extracorporeal cardiac shock wave (ECSW) therapy ameliorates myocardial ischemia in animal models of myocardial infarction[1] and in patients with chronic refractory angina pectoris.[2] The precise mechanisms of its action and its influence on cardiac tissue, however, remain controversial3. The goal of the present study was to evaluate the effects of SW application on adult rat heart *in vivo*, with respect to cardiac stem/primitive cell apoptosis proliferation, and differentiation. 4-month-old Fisher 344 male rats were subjected to ECSW therapy with 100 shots at energy flux density 0.25 mJ/mm^2 3 times over 1 week. Control and treated rats were sacrificed at week 5 and 13 of the study; hearts were arrested in diastole, excised and fixed in formalin. Successively, paraffin embedded sections were examined by histochemistry, immunohistochemistry or immunofluorescence. The hematoxylin and eosin staining revealed no differences in terms of fibrosis and inflammation. Masson's trichrome and Sirius Red stain confirmed the absence of fibrosis and unchanged extracellular matrix collagen content at all time points. The percentage of apoptotic cells (identified by TUNEL) was similar at 5 weeks (0.33±0.019% control *vs* 0.37±0.037% treated,

n=4, P=0.35) and at 13 weeks (0.35±0.024% control *vs* 0.39±0.023% treated, n=4, P=0.28). Immunohistochemical analysis of rat myocardium at 13 weeks after SW treatment revealed the presence of orthochromatic c-kit(+) cell clusters, while only single sparse c-kit(+) cells were observed in the control hearts. In the treated hearts, 55.7±1.34% of cardiac primitive cells were Ki67(+), while in the control hearts the cycling cells represented 35.74±2.93% (n=4, P<0.05). Cell clusters included FLK-1(+) endothelial progenitor cells and, accordingly, the number of arterioles was 1.12-fold higher in the treated hearts (n=4, P<0.05). In conclusion, the ECSW therapy is an effective way of cardiac stem cell and cardiac tissue regeneration activation.

References

1. Uwatoku T et al. Coron Artery Dis. 2007;18:397-404.
2. Khattab AA et al. Int J Cardiol 2007;121:84-5.
3. Jargin SV. Int J Cardiol 2010;144:116-7.

SB431542 ALTERS THE HISTORY OF MYELOFIBROSIS IN GATA1^{low} MICE

M. Zingariello,[1] L. Sancillo,[2] B. Ghinassi,[3] D. Bosco,[4] A.R. Migliaccio,[3] R.A. Rana[2]

[1]Campus Biomedico, Faculty of Medicine, Rome; [2]Department of Medicine and Aging Sciences, Section of Human Morphology, University "G. d'Annunzio" Chieti-Pescara; [3] Mount Sinai School of Medicine, NY, USA; [4]IGM CNR, Chieti, Italy.

The marrow in primary myelofibrosis and mouse models of the disease is characterized by increased levels of cytokines which regulate hematopoiesis including TGF-β.To clarify the role of TGF-β in development of myelofibrosis in the Gata1^{low} mouse model, this factor in plasma and marrow were measured. Gata1^{low} mice express normal levels of TGF-β in plasma and levels of TGF-β mRNA and protein only 2-times greater than normal in marrow. By immunoelectron microscopy,patchy TGF-β deposits associated with collagen fibers were observed in the marrow of mutant mice indicating that fibrosis may contribute to disease progression also in Gata1^{low} mice.To evaluate whether inhibition of TGF-β signaling would ameliorate myelofibrosis in Gata1^{low} mice,were treated with SB431542, a small inhibitor of TGF-β,that prevent renal fibrosis in mice. 6 males and 6 females were treated with SB431542 as described for renal fibrosis. Treatment was well tolerated and the SB43542-treated mice were easily recognized by being more active and with shinier coats. At the end of the 4th cycle, mice were sacrificed and analyzed. The treatment did not affected hematocrit levels but drastically reduced the number of circulating poikylocytes. Treatment increased total cell number and frequency of erythroid cells but not of MK in the femur. Fibrosis and microvessel density were reduced. Increased Mallory staining of bones was observed but the femur became resistant to fracture, suggesting that overall bone structure improved. The treatment reduced spleen weight and cell numbers, the frequency of erythroid cells and MK in the organ remained hight, overall hematopoiesis in spleen was reduced. SB43542-treatment restored the morphological appearance of the liver and reduced the frequency of MK. SB431542 treatment alters the natural history of myelofibrosis in Gata1^{low} mice.

INVOLVEMENT OF NF-κB/IκBα PATHWAY IN TRAIL RESISTANCE OF HUMAN ERYTHROBLASTS

S. Sancilio, V. di Giacomo, L. Centurione, M.A. Centurione, R. Di Pietro

Department of Medicine and Science of Aging, University G. d'Annunzio Chieti-Pescara, Chieti, Italy.
E-mail: r.dipietro@unich.it

The biological activity of TNF-related apoptosis inducing ligand (TRAIL) was analyzed on primary human erythroblasts derived from mononuclear cells of blood donors, kept in culture in the presence of 20% foetal calf serum, growth factors (EPO, SCF, IL-3) and glucocorticoids (10^{-6} Dexamethasone, 10^{-6} Oestradiol) or under growth factor and serum starvation. In the presence of growth factors and serum, primary erythroblasts showed a differential expression of TRAIL-receptors (Rs) at various degrees of maturation and responded to TRAIL treatment with a mild cytotoxicity. Instead, in the absence of serum and growth factors, TRAIL treatment unexpectedly upregulated TRAIL-R4 decoy receptor and promoted erythroblasts survival. The concomitant activation of NF-κB/IκB survival pathway was detected with Western blotting and immunofluorescence procedures and confirmed by experiments performed with SN50, a pharmacological inhibitor of the NF-κB/IκB pathway. Our study indicates that TRAIL has a twofold activity on erythroid lineages since it mediates eythroid cells apoptosis in the presence of serum and growth factors, while it promotes erythroid cells survival through the activation of the NF-κB/IκB pathway under starvation conditions.

VEGF INDUCES HUMAN ENDOTHELIAL PROGENITOR CELLS TO PROLIFERATE BY TRIGGERING OSCILLATIONS IN [Ca^{2+}]i

G. Guerra,[1] M. Cinelli,[4] S. Montagnani,[4] S. Dragoni,[2] U. Laforenza,[2] V. Rosti,[3] F. Tanzi,[2] F. Moccia[2]

[1]Dept. of Health Sciences, University of Molise, Campobasso, Italy; [2]Dept. of Physiology, University of Pavia, Pavia; [3]Laboratory of Clinical Epidemiology, Fondazione IRCSS Salvatore Maugeri, Pavia; [4]Biomorphological and Functional Sciences Dept., University of Naples Federico II, Italy. E-mail: germano.guerra@unimol.it

Endothelial progenitor cells (EPCs) traffic from the bone marrow to the site of tissue regeneration and sustain neo-vascularization after acute vascular injury and upon the angiogenic switch in solid tumors. Therefore, they represent a suitable tool for cell-based therapy in regenerative medicine and provide a novel promising target in the fight against cancer. The main stimulus responsible for EPC egression from the bone marrow and engraftment within neovessels is vascular endothelial growth factor (VEGF). Intracellular Ca^{2+} signals regulate numerous endothelial functions, such as proliferation, migration, and differentiation, and underpin VEGF effect on mature endothelium. We have recently shown that EPC growth is governed by a store-dependent Ca^{2+} entry (SOCE) pathway on the plasma membrane, which is activated by depletion of the inositol-1,4,5-trisphosphate ($InsP_3$)-sensitive Ca^{2+} pools.[1] The present study aimed at investigating the nature and the role of VEGF-elicited Ca^{2+} signals in EPCs. All the putative SOCE mediators (i.e. TRPC1, TRPC4, Orai1 and Stim1) were present in EPCs. VEGF induced long lasting Ca^{2+} oscillations, however, removal of external Ca^{2+} ($0Ca^{2+}$) and SOCE inhibition with BTP-2 reduced the number of Ca^{2+} spikes. Blockade of phospholipase C-γ (PLC-γ) with U73122 and emptying the $InsP_3$-sensitive Ca^{2+} pools with cyclopiazonic acid (CPA) prevented the Ca^{2+} response to VEGF. Accordingly, the Ca^{2+} response to VEGF was inhibited by superfusing CPA during the ongoing

oscillations. Notably, VEGF induced EPC was abrogated by SOCE inhibition with BTP-2. Similarly, VEGF promoted NF-kB translocation into the nucleus in a BTP-2-sensitive manner. Thus, VEGF causes an initial $InsP_3$-dependent Ca^{2+} discharge followed by SOCE-mediated Ca^{2+} entry in cEPCs. SOCE, in turn, controls store refilling and induces cell proliferation by recruiting NF-kB.

Reference

1. Sánchez-Hernández Y et al. Stem Cells Dev 2010;19:1967.

RUNX/SMADS INTERACTION IS IMPAIRED IN OSTEOBLASTS FROM Fgf2-/- MICE

M.G. Sabbieti,[1] D. Agas,[1] M.M. Hurley,[2] L. Xiao,[2] L. Marchetti[1]

[1]School of Bioscience and Biotechnology, University of Camerino, Camerino, Italy; [2]Department of Medicine, University of Connecticut Health Center, Farmington, Connecticut, USA. E-mail: giovanna.sabbieti@unicam.it

Bone morphogenetic protein 2 (BMP2) is one of the most potent regulator of osteoblast differentiation and bone formation. R-Smads (Smads 1/5/8) are the major transducers for BMPs receptors and, once activated, they translocated in the nucleus regulating transcription target genes by interacting with various transcription factors.[1] Runx2 proteins have been shown to interact through their C-terminal segment with Smads and this interaction is required for *in vivo* osteogenesis.[2] In particular, recruitment of Smads to intranuclear sites is Runx2 dependent, and Runx2 factor may accommodate the dynamic targeting of signal transducer to active transcription sites.[3] Previously, we have shown, by *in vitro* and *in vivo* experiments, that BMP2 up-regulated FGF-2 which is important for the maximal responses of BMP-2 in bone.[4] Now, by biochemical, immunofluorescence and immunoelectron microscopy approaches, we found that BPM2 was also able to induce nuclear accumulation and colocalization of Runx2 and Smads1/5/8 in presence of endogenous FGF-2, while Runx/Smads nuclear interaction was markedly reduced in Fgf2-/- osteoblasts. Based on these preliminary data, we hypothesize that the impaired nuclear accumulation of Runx2 in Fgf2-/- osteoblasts could reduce R-Smads sub-nuclear targeting with a consequent decreased expression of differentiating markers and impaired bone formation in Fgf2 null mice.

References

1. Miyazono K et al., Cytokine Growth Factor Rev 2005;16:251-263.
2. Choi JY et al., Proc Natl Acad Sci USA 2001;98:8650-5.
3. Zaidi SK et al., Proc Natl Acad Sci USA 2001;99:8048-53.
4. Naganawa T et al., J Cell Biochem 2008;103:1975-18.

AGE-RELATED CHANGES OF THE GLICOSAMINOGLYCANS IN THE ANTERIOR CHAMBER ANGLE OF THE HUMAN EYE

C. Cavallotti,[1] E. De Santis,[1] M. Sabbatini[2]

[1]Dept. of Human Anatomy, University "La Sapienza" Rome, Rome; [2]Department of Clinical and Experimental Medicine, Laboratory of Human Anatomy, University of Eastern Piedmont "A. Avogadro" Novara, Italy. E-mail: cavallotti@uniroma1.it

Morphological, histochemical, morphometrical, biochemical and/or age-related changes in glycosaminoglycans of the anterior chamber angle of the human eye were evaluated. This evaluation let us to shortly review the recent results on old molecules such as mucous-proteins today said glycosaminoglycans. Small human samples were drawn, after death, by mean of a small biopsy from 24 eyes of young and old humans, without any aesthetic damage for enrolled people. Samples harvested from tissues localized around the anterior chamber angle of the eye were divided in four fragments, each used for morphological, histochemical, ultra-structural and biochemical analysis. Quantitative analysis of images was performed to evaluate morphometric data that were compared with biochemical ones. All results were statistically analyzed. Our findings demonstrate the following results on the glycosaminoglycans contained in the anterior chamber angle: 1) deposition of fibrous granular material with increasing age; 2) increased electron density of these structures ; 3) strong decrease of hyaluronic acid content; 4) increase of glycosamino-glycans. In conclusion glycosaminoglycans of the cells localized near the human anterior chamber angle undergo age-related changes, as demonstrated by morphological, histochemical, morphometrical and biochemical results. The present results let us to shortly evaluate the presence of glycosamino-glycans in the anterior chamber angle of the human eye where these substances control the normal outflow resistance.

HETEROGENEITY OF CELL GLYCOCONJUGATES OCCURRING IN THE HORSE GUTTURAL POUCHES: A HISTOCHEMICAL PERSPECTIVE

F. Parillo,[1] M. Zerani,[1] G. Catone,[1] F. Mignini[2]

[1]School of Veterinary Medicine Science and [2]School of Pharmacy, University of Camerino, Italy. E-mail: francesco.parillo@unicam.it

We investigated the carbohydrate composition of the mucosubstances present in the equine guttural pouches using conventional histochemical reactions in conjunction with glycolitic digestions and lectins combined with chemical and enzymatic deglycosylation pretreatments. The goblet cells produced chondroitinsulphate B, heparin, heparan sulphate and sialic acid residues. The acinar cells also expressed these substances, except for heparin, whereas ductal cells produce chondroitinsulphate B and sialic acid. Neutral sugars were also found in each cell type. Glycosoaminoglycans provide a hydrophilic environment which desiccation of the guttural membranes during air passage and may be involved in the pathogenesis of some bacterial disease, such as equine strangles. Lectin histochemistry revealed in the goblet cells the occurrence of O- and N-linked glycans with α-Fuc and GlcNAc whereas β-GalNAc, β-Gal-(1-3)-GalNAc, β-Gal-(1-4)-GlcNAc and α–Gal belonged to O-linked glycoproteins. The acinar and ductal cells expressed α-Man/α-Glc in N-linked glycans, GlcNAc in O- and N-glycoproteins and β-GalNAc, β-Gal, α–Gal in O-linked glycoproteins. The Golgi area of the epithelial cells expressed α-Fuc in O-linked glycans, GlcNAc in N-linked glycans and sialic acid linked to β-GalNAc and β-Gal. Sulphocarbohydrates and sialic acids (α2,3-6), linked to-α/β-Gal and sialic acids (α2-6) linked to β-GalNAc, were also demonstrated. Such diversity of the mucin saccharide residues may be implicated in the binding of macromolecules such as bacteria or viruses, thus playing a role in the organism's host-defense mechanism in the guttural pouches.

INVESTIGATIONS ON THE MUCOCYTES OF THE MANTLE AND FOOT SKIN OF *JANTHINA PALLIDA* MOLL. GAST. PROS.

C. Calabrò,[1] C. Bertuccio,[2] D. Palombieri,[3] P. LoCascio[3]

[1]Dept.of Animal Biology and Marine Ecology, [2]Dept.of Life Science Malpighi, [3]Dept. of Food and Envir. Science,Univ.of Messina, Italy. E-mail: deborahpalombieri@alice.it

Previous studies on the skin of several species of Gastropoda

Prosobranchia Mollusca, revealed mucous cells vary in number, localization and chemical constitution.[1,2,3] The aim of this study was the individuation of mucocytes in the mantle and foot epithelia, the histochemical and immunohistochemical characterization of mucus and its functional role. The mantle skin consists of a simple external epithelium with cylindrical pigmented ciliated cells and mucous cells, and internal epithelium characterized by cubic cells with a thin cuticle and intraepithelial and intraconnectival mucocytes. The foot has a conic shape and it is small without operculum, since *Janthina pallida* is floating on the water-surface by means of peculiar rafts made of air bubbles and mucus. The skin consists of simple dorsal epithelium with cylindrical cells and mucous cells, and of ventral simple epithelium with cubic and mucous cells. Both in the mantle or in the foot there are mucocytes different for chemical composition. The presence in the mucous secretion of glicoproteins, proteoglycans with sulphate and with carboxylic groups, glycoconjugate as αDglucose, αDmannose, αLFucose and βDgalactose, may be related to the role these molecules play in the recognition and elimination of pathogens. nNOs immunopositivity of nervous fibers innervating mucocites of mantle is related to NO function stimulating secretion. The presence of NO in mucous cells of the foot is considered to be involved in the modulation of the mucus secretion with paracrine activity and in the cell-cell communication.

References

Bolognani Fantin A.M.,Ottaviani E. Histochem J 1990;22:475-9.
Licata et al. Acta Histochem 2003;105:143-9.
Calabrò et al., Folia Histochem et Cytobiol 2005;43:151-6.

ALTERED LECTIN-BINDING PATTERN OF EFFERENT DUCTS IN A TRICOLOR CAT AFFECTED BY KLINEFELTER SYNDROME

G. Ventriglia,[1] G. Accogli,[1] L. Valentini,[1] S. Desantis[1]

[1]*Dept. of Animal Production, University of Bari Aldo Moro, Italy. E-mail: g.ventriglia@veterinaria.uniba.it*

Recent investigations[1] indicate that cat efferent ducts (EDs) play a role in reabsorption of the fluid and proteins leaving the testes, dependent also on androgen.[2] The males with Klinefelter syndrome (XXY) show a variable degree of androgen deficit responsible for testicular disfunction.[3] Since we demonstrated synthesis and secretion of glycoconjugates in normal cat EDs1, here we investigated the glycoprotein pattern of the EDs from a tricolor cat with Klinefelter syndrome (39,XXY), by means of the lectin histochemistry, utilizing a panel of 12 lectins in association with sialidase (s) treatment. Cilia of ciliated cells reacted with HPA, SBA, Con A, KOH-s-WGA, GSA II in normal cats and with MAL II, SNA, Con A and KOH-s-WGA in XXY cat. The luminal surface of non-ciliated cells bound MAL II, KOH-s-PNA, Con A in all samples, RCA_{120} and HPA only in normal subjects and PNA in XXY cat. The supra-nuclear cytoplasm of non-ciliated cells expressed SNA and Con A affinity in XXY cat and also MAL II, KOH-s-PNA, RCA_{120}, SBA in normal cats. These results indicate that negative charges are mainly expressed on the cilia of XXY cat EDs, whereas a more complex glycoconjugate pattern, probably related to an more effective endocytotic apparatus, is expressed in the supranuclear cytoplasm of non-ciliated cells from normal EDs.

References

1. Arrighi S et al. Histol. Histopathol 2001;25:433-44.
2. Jones RC. The epididymis: From molecule to clinical practice. Robaire and Hinton eds, Kluwer Academic/Plenum Publ 2002;11-33.
3. Wikström AM and Dunkel L. Horm. Res. 2008;69:317-26.

IN VITRO MATURATION INDUCES GLYCOCONJUGATE CHANGES IN EQUINE CUMULUS-OOCYTE COMPLEXES

G. Accogli,[1] M.E. Dell'Aquila,[1] M. Filioli Uranio,[1] S. Desantis[1]

[1]*Dept. of Animal Production, University of Bari Aldo Moro, Italy. E-mail: gianluca. accogli@uniba.it*

In vitro matured oocytes (IVM) suffer some inadequacies when compared with *in vivo* matured ones.[1] Inadequate IVM can yield under- or overmature oocytes, which will not undergo normal fertilization and embryo development.[2] Glycoconjugates play a key role in oocyte maturation, and in oocyte-sperm interactions leading to fertilization,[3,4] thus the knowledge of oligosaccharide pattern of equine COCs could provide useful information about the comparison between immature and matured COCs. Cumulus enclosed oocytes from abbattoir ovaries were fixed in Bouin's solution and embedded in paraffin wax either before or after IVM. Sections were stained with 13 lectin (MAL II, SNA, PNA, DBA, RCA_{120}, SBA, HPA, Con A, WGA, GSA I-B4, GSA II, UEA I, LTA). The radiata zone of immature COCs reacted with all used lectins, whereas matured COCs stained with MAL II, SNA, HPA, SBA, and Con A. The zona pellucida of both COCs types bound MAL II, SNA, SBA, and Con A, whereas immature COCs reacted also with RCA_{120}, WGA, and matured ones stained with UEA I. The ooplasm of both types of COCs reacted with HPA, Con A, GSA II, UEA I and LTA, whereas immature oocytes bound also SNA, SBA, WGA, GSA I-B_4. These results indicate that IVM modifies glycoprotein pattern of equine COCs and prompted us to undergo further studies to investigate the role of the modified oligosaccharides in oocyte viability, capacity to undergo fertilization and normal embryonic development.

References

1. Deleuze S. et al. Theriogenology. 2009, 72:203-9.
2. Hinrichs K. & Di Giorgio L.M. J Reprod Fertil Suppl 1991, 44:369-74.
3. Dell A. et al. Biochim Biophys Acta 1999;1473:196-205.
4. Clark G.F. & Dell A. J. Biol Chem 2006;281:13853-6.

HEPARAN SULPHATE SUGAR CHAINS ARE INVOLVED IN PGF2α-INDUCED OSTEOBLAST GROWTH AND DIFFERENTIATION

L. Marchetti, D. Agas, M.G. Sabbieti

School of Bioscience and Biotechnology, University of Camerino, Camerino, Italy.
E-mail: luigi.marchetti@unicam.it

Heparan sulfate proteoglycans (HSPGs) consist of a protein core and heparan sulfate (HS) glycosaminoglycan chains which confer structural and functional diversity.[1] HSPGs are required for fibroblast growth factor (FGF) binding to its tyrosine kinase receptor. They are also considered dynamic regulators of FGF signaling.[2] The role of PGF2α as modulator of HSPGs, FGF-2 and FGF receptors (FGFRs) was evaluated in order to better understand PGF2α-mediated signalling in osteoblast metabolism. The novel observation that PGF2α was able to promote the formation of HSPGs/FGF-2/FGFRs complexes is hereby depicted. Our data also suggested that PGF2α could induce new synthesis of HS chains on osteoblasts by a mechanism involving a modulation of MAPK signaling and that HS is required for the regulation of FGF-2 induced by PGF2α. In particular, we showed that HSPGs are necessary for the expression of phospho-p44/42, since their proteolytic cleavage before PGF2α administration down-regulated phospho-p44/42 basal expression, likely inhibiting FGFRs tyrosine kinase activity. Interestingly, MAPK signalling influenced syntheses and

localization of FGF-2, its specific receptor and HS. In addition, HSPGs proteolytic cleavage and MAPK kinase inhibition also revealed that PGF2α–induced cell proliferation is dependent on HSPGs and FGF-2 specific receptor, respectively. Of further relevance of this study, we demonstrated, by using a specific siRNA for FGFR1, that PGF2α modulates Runx2 expression by FGFR1 and HS.

References

1. Turnbull J et al., Trends Cell Biol 2001, 11:75-82.
2. Mundhenke C et al., Am J Pathol 2002, 160:185-94

GLYCOCONJUGATES OF THE TROUT AND SOLE INTESTINAL MUCOSA FOLLOWING *IN VITRO* EXPOSURE TO PROBIOTIC BACTERIA

M.G. Gabrielli, D. Tomassoni, S. Panarello, M. Coman, D. Accili

School of Biosciences and Biotechnology, University of Camerino, Italy

Probiotic bacteria are known to exert several beneficial effects such as nutritional contribution and protection against pathogens, either by the production of antimicrobial compounds or through competition for mucosa binding sites. Their effect on development and/or activation of beneficial immune responses in the host is widely recognized although not fully clarified. The use of probiotics has been applied in human and animal nutrition with successful results. In order to investigate a possible relation between probiotic bacteria and glycosylation pattern expressed by the intestinal mucosa, we studied the binding patterns of several lectins in the lining epithelium of trout and sole intestine exposed to differently modified microbiotica environment. Three experimental treatments were realized by *in vitro* exposure of fish intestines to probiotic bacteria (*L. rhamnosus*, and *L. paracasei*, group 1), pathogen bacteria (*V. anguillarum,* group 2), probiotic bacteria followed by pathogens (group 3). In the control group (group 4), the intestines were exposed to sterile saline solution and TSBgs in the same conditions, as above. The preliminary results indicate that the exposure of the sole intestine to probiotic bacteria affects the lectin binding patterns of specific sugar residues and modulates the effect induced by exposure to the pathogens. In the trout intestinal mucosa, interesting data were also obtained accounting for a relation between probiotics and modified expression pattern of the ladderlectin and intelectin, both proposed to function in mucosal and cellular innate immune defence against microrganisms.

BIOLOGICAL ACTIVITY OF ANTIOXIDANTS IN EXPERIMENTAL MODEL OF *C. AURATUS*: MORPHOLOGICAL STUDY

A. Alesci, G. Silvestri, A. De Pasquale, D. Palombieri, E.R. Lauriano, S. Pergolizzi

Dept of Food and Environmental Sciences, University of Messina, Italy. E-mail: alesci@live.it

Antioxidants are substances, that protect from oxidative damages and chronic diseases.[1] This study aims to evaluate the effects of polyphenols from *Olea europea*, and carotenoids (astaxanthin) from *Phaffia rhodozyma* on diseases to accumulation in organs of *C. auratus.* Two experiments are conducted: the animals are divided into 2 groups: 1) with induced diseases by lipid accumulation, 2) treated with absolute ethanol. These groups are subdivided into 2 subgroups, respectively: curative treatment with 1) polyphenols 2) astaxanthin. Intestine (polyphenols group). The results show an increase in the hypercholesterolemic group of circulating macrophages, due to oxidation of LDL, become foam cells typical of the formation of atherogenic plaques, which express specific receptors for attachment to the intimate endothelial and atheroma formation.[2] In the curative group these cells disappear, returning to assume normal morphology and show lipochrome carotenoid pigment that is believed, preventing the ox-LDL process.[3] Intestine (alcoholic group). The results show, in the alcohol group, an increase of cells with mucous hypersecretion. Also disorganization of the parenchyma and cellular congestion appear. In contrast, the group fed with Phaffia rhodozyma in addition shows a normal morphology, with greater homogeneity of the parenchyma[4] with evident beneficial effect of the carotenoid pigment astaxanthin as antagonist of alcohol-induced damage.

References

1. Lü JM et al., J Cell Mol Med 2010;14:840-60.
2. Park DW et al., Ex and Molecular Med 2009;3:171-9.
3. Tyagi A et al., Cancer Prev Res (Phila). 2009 ;2(1):74-83
4. Xiao AF et al., W J of Microbiology and Biotechnology 2009;11: 2029-34.

PRELIMINARY DATA OF C2C12 CELL DEATH INDUCED BY PHYSICAL AGENTS

V. Baldassarri,[1] S. Salucci,[1] M. Battistelli,[1] S. Burattini,[1] B. Canonico,[1] S. Papa,[1] E. Falcieri[1,2]

[1]Dep. of Earth, Life and Environmental Sciences, Urbino University "Carlo Bo" and [2]IGM, CNR, IOR, Bologna, Italy. E-mail: valentina.baldassarri@uniurb.it

Apoptosis is essential for skeletal muscle development and plays a pivotal role in maintaining its homeostasis, both in undifferentiated and differentiated conditions.[1,2] In addition, it has been frequently demonstrated in several muscle myopathies[3] and in sarcopenia, as well as in denervation and disuse. In this work we have investigated skeletal muscle cell death, induced *in vitro* by a variety of physical triggers, and analysed by TUNEL reaction, electron microscopy and flow cytometry. For this purpose, C2C12 myoblasts and myotubes, were exposed to UV-B (312 nm) for 30 min, low pH (4-6) for 3h, hyperthermia for 1h at 45-50°C and hypothermia for 4h at 0-6°C, all followed by 2-4h recovery. C2C12 murine myoblasts and myotubes show morphological apoptotic changes after UV-B irradiation. In myoblasts, a characteristic chromatin margination at nuclear periphery frequently appears. Even if cup-shaped masses, comparable to those of more classic apoptotic models, can not be found, nuclear features, when analysed both by electron microscopy and TUNEL, appear to suggest apoptosis.[1] Low pH induces apoptosis, but also necrosis, both characterized by empty nuclei and vacuolated cytoplasm. Hyperthermia, known as a powerful apoptotic inducer in hemopoietic cells, as well as hypothermia, appear almost exclusively necrotic trigger both in undifferentiated and differentiated cells. As for chemical cell death inducers, skeletal muscle cells seem to be sensitive to physical agents in a trigger-dependent way.

References

1. D'Emilio A., et al., Histol. Histopathol 2010;25:21.
2. Salucci S., et al., Micron, 2010.
3. Loro E et al., Cell death differ 2010;17:1315.

PROTECTIVE ACTION OF HYDROXYTYROSOL DERIVATIVES *vs* APOPTOSIS: A PRELIMINARY STUDY

S. Salucci,[1] V. Baldassarri,[1] S. Burattini,[1] A. Accorsi,[2] E. Piatti,[2] G. Zappia,[2] G. Piersanti,[2] J.L. Espartero Sanchez,[3] E. Falcieri[1,4]

[1]Dept. Earth, Life Environm. Sciences; [2]Dept. Biomol. Sciences, Urbino Univ. "Carlo Bo"; [3]Faculty of Pharmacy, Univ. De Seville; [4]IGM, CNR, IOR, Bologna. Italy. E-mail: sabrina.burattini@uniurb.it

Hydroxytyrosol (HT), is released primarily in olive mill wastewater and in olive oil.[1] In animal and cellular model studies, HT and its metabolites showed strong antioxidant and antimicrobial properties, as well beneficial effects on the cardio-vascular system and in several human diseases.[2] Differently, many reports in tumor cells,[3] suggested that HT down-regulates cell viability, and proliferation, and induces apoptosis. Nevertheless, little is known about its effect on normal cell apoptosis. Previous biochemical studies suggested that HT and HT derivatives, in particular the HT-Laurate (HT-L), showed antioxidants effects on human erythrocytes.[4] Here, we have investigated the effect of 20 μm HT and 5 μM HT-L in C2C12 myoblasts and leukemic U937 cells after H_2O_2-treatment. Cell response was characterized by microscopy. HT and HT-L, added to the control cells, do not influence cell viability. On the other hand, H_2O_2-treated C2C12 and U937 cells, at the proper H_2O_2 concentrations,[5] show a number of apoptotic features.[6] Their pre-incubation with HT and HT-L prevent cell death, in particular HT-L-treatment. The anti-apoptotic action of these compounds, both on normal myoblasts and on tumor cells, suggested by this preliminary study, could represent an interesting property with potential biological and clinical applications.

References

1. Fabiani R et al. J Nutr 2008;138;1411.
2. Goya L et al. Eur J Nutr 2007;46;70.
3. Han J et al. Cytotechnol 2009;59;45.
4. Accorsi A et al. Food Chemistry, Submitted.
5. Salucci et al. Micron 2010;41;966.
6. D' Emilio et al. Histol Histopathol 2010;25;21.

CYTOTOXICITY INDUCED BY NONYLPHENOL IN GASTRIC EPITHELIAL CELL LINE

L. Manente,[1] A. Lucariello,[1] A. Perna,[1] A. Sellitti,[2] C. Costanzo,[1] V. Laforgia,[2] I. Cavallotti,[1] M. De Falco,[2] A. De Luca[1]

[1]Dept. of Medicine and Public Health, Section of Human Anatomy, Second University of Naples; [2] Dept. of Biological Sciences, Section of Evolutionary and Comparative Biology, University of Naples Federico II, Italy. E-mail: antonio.deluca@unina2.it

Nonylphenol (NP) is a lipophilic and estrogenic compound that bioaccumulates in aquatic and terrestrial organisms so entering the food chain. Its toxic and estrogenic activity has been investigated in many *in vitro* and *in vivo* studies. Human exposure to NP may occur by cutaneous absorption, ingestion of contaminated food or water and inhalation. Although the cytotoxic effects of NP have been studied, its effects on cell death and related mechanisms are unknown. Drawing from this background, the aim of this study was to investigate NP effects on gastric epithelial cell line (AGS). Cell cycle was analyzed by flow cytometry, p21 and p27 induction; apoptosis was analyzed by flow cytometry and Annexin-V assay, Fas, FasL, caspase-8, and caspase-3 activation. We demonstrated that NP affected cell cycle and apoptosis in a time- and dose-dependent manner, reaching the best effect at concentration of 10^{-7}M for 48 h. Flow cytometry revealed that treatment with 10^{-7}M NP led to the accumulation of cells at the G_2/M transition and increased the population of apoptotic cells. Moreover, the 10^{-7}M concentration induced a marked increase of Fas and FasL expression and the activation of caspase 8 and 3 but not the activation of caspase 9. In conclusion we demonstrate for the first time that NP induces apoptosis in AGS cells in a dose-dependent manner with involvement of Fas and FasL pathway. These findings offer new perspectives to understand the fundamental mechanism of chemical-induced apoptosis in AGS cells.

CELL DEATH INDUCED BY PLATINUM COMPOUNDS IN B50 NEUROBLASTOMA RAT CELLS

M.G. Bottone,[1,2] G. Santin,[1] V.M. Piccolini,[1] P. Veneroni,[1] S. Barni,[3] V. Dal Bo,[1] F.P. Fanizzi,[4] G. Bernocchi[1,2]

[1]Dept. of Animal Biology, Lab. of Cell Biology and Neurobiology; [2]CNR Inst. of Molecular Genetics, Histochemistry and Cytometry Section; [3]Dept. of Animal Biology, Lab. of Comparative Anatomy and Cytology. University of Pavia; [4]Dept of Biological and Environmental Sciences and Technologies, University of Lecce; Italy. E-mail: bottone@unipv.it

Cisplatin (cisPt) is one of the most active chemotherapeutic agents used in the treatment of childhood and adult malignancies. CisPt induces cell death through different pathways. Although its effectiveness, the continued clinical use of cisPt is limited by onset of severe side effects (nephrotoxicity, ototoxicity and neurotoxicity) and drug resistance. Therefore, the search for new platinum-based drugs is one of the main topics in experimental oncology. One of the adopted strategies is the synthesis of platinum compounds able to form different types of Pt-DNA adducts or to react with other subcellular targets. In this context, [Pt(*O,O'*-acac)(gamma-acac)(DMS)] (PtAcac DMS), which reacts preferentially with protein thiols or thioethers. In our research, different approaches were used to compare cisPt and PtAcacDMS on rat neuroblastoma cells. Analysis were performed both to compare the ability of acac and cisPt to cause cell death and to investigate the intracellular mechanisms of cytotoxicity induced by these platinum compounds. These compounds cause apoptosis triggering the mitochondrial pathway, however the apoptotic cell percentage after PtAcacDMS administration was higher than after cisPt. Flow cytometry analysis have demonstrated that this compound exerts a cytostatic action. *In vitro*, PtAcacDMS seems to have less neurotoxic effects than cisPt, showing antineoplastic effectiveness.

Grant: Fondazione Banca del Monte di Lombardia.

MELATONIN PROTECTS HT22 CELLS FROM OKADAIC ACID-INDUCED CELL DEATH

F. Luchetti, B. Canonico, P. Ferri, M. Arcangeletti, E. Cesarini, L. Galli, F. Buccella, L. Zamai, S. Papa

Dept. of Earth Sciences, Life and Environment, University of Urbino "Carlo Bo", Italy. E-mail: francesca.luchetti@uniurb.it

The production of reactive oxygen species (ROS) and their detoxification are normal physiological processes, but an imbalance between the production of ROS and their removal may lead to oxidative stress. Neurodegenerative diseases, such as Alzheimer disease (AD), Parkinson disease and amyotrophic lateral sclerosis,[1] are associated with increased oxidative stress (OS). Neuronal mitochondria are vulnerable to OS due to their role in energy supply and use, causing a possible production of giant and/or young mitochondria with compromised DNA. Okadaic acid (OA) is a phosphatase inhibitor that

induces hyperphosphorylation of Tau proteins, a major hallmark in AD. Our previous data demonstrated that melatonin has protective effect in the intrinsic apoptotic pathway[2] in U937 cells, here we'll document its neuroprotective effects on HT22 cells treated with OA. Our preliminary data show that OA strongly induces apoptosis (50%) and the treatment with different concentrations of melatonin partially prevent this phenomenon. Furthermore, data on mitochondria membrane potential (MMP) demonstrated melatonin ability to almost completely restore MMP, especially for melatonin 1-10 μM. To better characterize the neuroprotective role of melatonin at mitochondria level, we performed an immunostaining of 8-hydroxydeoxyguanosine. In conclusion our results suggest that melatonin is efficient in apoptosis protection and MMP restoration in this murine model and could be thought as an efficient substance to support pharmacological therapies in neurodegenerative disorders.

References

1. Clement AB et al. Free Rad Biol Med 2010;48:1236-41.
2. F. Luchetti et al. Free Rad Biol Med 2009;46:339-51.

BIOACCUMULATION OF CADMIUM AND ITS EFFECT ON GFAP (+) STRUCTURES IN ZEBRAFISH BRAIN

R. Favorito,[1] M. C. Grimaldi,[1] S. De Bonis,[2] M. Figliolia,[1] L. Cristino,[3] I. Ferrandino[1]

[1]Dept. of Biological Sciences, University Federico II of Naples; [2]Dept. of Structural and Functional Biology, University Federico II of Naples; [3]Inst. of Cybernetics, CNR of Pozzuoli, Italy. E-mail: ida.ferrandino@unina.it

Water contamination by metal compounds is a worldwide environmental problem. Fish accumulate metals in higher concentrations in their tissues by environmental absorption by the gills and gut. The present report is an attempt to investigate the influence of cadmium exposure on the accumulation of this metal in zebrafish brain and the correlative expression of GFAP marker for the glial cells, the first of the brain parenchyma to encounter metals crossing the blood-brain barrier. Cadmium accumulation in brain was measured by APAT IRSA-CNR method, 3000 Section 3010 division. GFAP immunoreactivity was determined by ABC technique. 18 adult fish were exposed to contaminated water (1.0 mg/L of CdCl2). Groups of 6 fish were killed after 2, 7 and 16 days. Another 18 fish were kept in uncontaminated water as control animals. We reveled that cadmium accumulation increased over time; cadmium concentration in treated fish brain appeared to increase exponentially and it was maximum after 16 days (2 days after: 22.23±1.0 ppm; 7 days: 33.16±0.82 ppm; 16 days: 45.04± 1.02 ppm). Immunohistochemical detection of GFAP structures revealed a considerable decrease of the immunoreactive structures during the treatment. In particular, we observed this reduction of GFAP immunoreactivity in the area of the optic tectum, medulla and cerebellum. Our data following treatment with cadmium indicate that a correlation exists between decrement of GFAP expression and cadmium accumulation found in the brain. May be cadmium crosses the blood-brain-barrier and it accumulates in the brain, in particular in glial cells.

ALTERED EXPRESSION AND ACTIVITY OF DIPEPTIDYLPEPTIDASE-IV IN THE LIVER OF A RAT MODEL OF OBESITY

E. Tarantola,[1] V. Bertone,[1] E. Capelli,[1] A. Ferrigno,[2] M. Vairetti,[2] U. Cillo,[3] S. Barni,[1] I. Freitas[1,4]

[1]Dept of Animal Biology; [2]Dept of Internal Medicine; [4]IGM-CNR, Histochemistry and Cytometry Section; University of Pavia; [3]Dept. General Surgery and Organ Transplantation, University of Padua, Italy. Italy. E-mail: freitas@unipv.it

Biliary complications are considered the Achille's heel of liver transplantation. To evaluate its response to the various phases of transplantation specific markers are necessary. Given the scarcity of donors, fatty livers are currently being used as grafts, notwithstanding their poor tolerance to conventional cold preservation. Alkaline phosphatase activity was shown to be scarse in bile canaliculi of obese Zucker rats.[1] Dipeptidyl-peptidase-IV (DPP-IV) has been investigated as an alternative marker. DPP-IV plays an important role in the glucose metabolism, rapidly cleaving incretins resulting in an enhanced insulin secretion and decreased blood glucose concentration; DPP-IV inhibitors are used to treat Type II diabetes.[2] DPP-IV activity was demonstrated with an azocoupling method, protein expression with immunhistochemistry and gene expression with PCR. Wistar, lean and obese Zucker rats were used as models. Gene expression was present in all models. In Wistar and lean Zucker rat liver a strong activity was seen in all segments of the biliary tree and weak positivity in sinusoidal cells. By contrast, a strong reduction of activity and expression were seen in the obese Zucker rat liver, ascribed to the massive structural rearrangement of the parenchyma due to the lipid accumulation. This is in keeping with altered glucose metabolism alteration in this model. DPP-IV can therefore be used only to follow transplantation damage only to the upper segments of the biliary tree.

References

1. Bertone et al. EJH 2011;55:24-32.
2. Yang et al, Life Sciences 2007;81:272-9.

TOLL-LIKE RECEPTOR 2 AND LIPOPROTEIN ACCUMULATION IN MACROPHAGES OF A HYPERCHOLESTEROLEMIC *C. AURATUS*

E.R. Lauriano,[1] A. Alesci,[1] A. De Pasquale,[1] D. Zaccone,[1] A. Marino,[2] G. Silvestri,[3] S. Pergolizzi[1]

[1]Dept. of Foods and Environmental Sciences, [2]Dept. of An. Biol. and Mar. Ecol.,[3]Pharmaco-biological Dept., University of Messina, Italy. E-mail: alesci@live.it

Plasma lipoproteins and macrophages play critical roles in the initiation and progression of the atherosclerotic lesion, that is focused on the *foam cells* formation, lipid-loaded macrophage. Monocytes are recruited to the peripheral tissues by inflammatory processes (in part promoted by proinflammatory effects of oxidized LDL), where they differentiate into resident macrophages, that respond to chemotactic agents, are phagocytic, mediate microbicidal activity, and express a variety of genes relevant to atherogenesis.[1] The TLR family is a highly conserved mechanism of host defense that mediates phagocytosis and signaling. TLRs bind not only exsogenous but also endogenous ligands, as modified lipoprotein. Several TLRs (TLR-1, -2, and -4) are expressed by activated macrophages in human atherosclerotic lesions. Differentiated tissue macrophages use multiple and diverse mechanisms to internalize lipoproteins.[2] In this work we found that feeding *C. auratus.* a high-cholesterol diet, resulted a significant presence of foam cells in the intestinal tissues. The expression of TLR2 has been

found in foam cells suggesting a novel mechanism of lipid accumulation in macrophages via TLR2- dependent macropinocytosis. Endogenous ligand for TLR2, induced extensive membrane ruffling in macrophages and cell spreading, associated with intracellular vacuolization. Based on these findings, we hypothesized that the TLR2-mediated cytoskeletal rearrangements may quantitatively increase the rate of lipoprotein uptake by macrophages and thus accelerate foam cells formation.

References

1. DJ Rader et al. Cell Metabolism: 2005 vol.1
2. YI Miller et al. Trends Cell Metabolism. 2009;19:227-32

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF TOLL-LIKE RECEPTOR 2 IN GUT OF *C. AURATUS* FED WITH A HIGH-CHOLESTEROL DIET

E.R. Lauriano,[1] A. Alesci,[1] G. Silvestri,[1] D. Zaccone,[1] A. Marino,[2] A. De Pasquale,[3] S. Pergolizzi[1]

[1]Dept. of Foods and Environmental Sciences, [2]Dept. of An. Biol. and Mar. Ecol., [3]Pharmaco-biological Dept., University of Messina, Italy. E-mail: alesci@live.it

Toll-like receptors (TLRs) are a group of pattern recognition molecules that play a crucial role in innate immunity. TLRs recognize a variety of endogenous and exogenous ligands.[1] The structural conservation of the archaic TLR system suggests that also the regulation of the immune response might be similar in fish and mammals. TLR2 is implicated in signaling mechanisms that contribute to chronic inflammatory diseases, including atherosclerosis. To date, 17 different TLRs have been identified in more than a dozen different fish species, as fugu (*T. rubripes*) and zebrafish (*D. rerio*). The intestinal epithelium is an active participant in the mucosal immune response through its expression of proinflammatory genes, secretion of inflammatory cytokines, and recruitment of inflammatory cells.[2] In this study , we found that feeding goldfish (*C. auratus*) a high-cholesterol diet, resulted hypercholesterolemia, accompanied by lipid accumulation in macrophages, also known as foam cell formation, that is a key process during the development of atherosclerosis. TLR2 was present at the apical surface of polarized intestinal epithelial cells and in the *lamina propria*, composed of macrophages, lymphocytes and stromal cells. In the intestinal epithelial cells and in the lamina propria cells of the control fish the TLR2 was express at low level. The distribution of the TLR2 in gut of *C. auratus* is similar to that reported in mammalian and is involved in the activation and regulation of inflammatory and immune responses.

References

1. A Rebl et al. Vet Immunology and Immunopathology 2009 (in press)
2. B Alvarez et al. Vet. Res. 2008;39:13.

PACAP AND PACAP RECEPTORS IN ADIPOSE TISSUE OF RATS FED WITH HIGH FRUCTOSE DIET

S. Valiante,[1] R. Crescenzo,[2] A. Sellitti,[1] A. Capaldo,[1] S. Salamone,[1] S. Iossa,[2] V. Laforgia,[1] M. De Falco[1]

[1]Dept. of Biological Sciences, Section of Evolutionary and Comparative Biology, University of Naples Federico II, Naples; [2]Dept. of Biological Sciences, Section of Physiology, University of Naples Federico II, Naples, Italy. E-mail: valiante@unina.it

The pituitary adenylate cyclase-activating polypeptide (PACAP) is involved in the regulation of adipogenesis[1] and has lipolytic effects on rat primary adipocytes.[2] PACAP acts through the binding of three receptors: PAC-1, VPAC-1 and VPAC-2.[3] PACAP receptors are expressed in human adipose tissue and rat adipocytes.[4] Controversial data are available about VPAC-1 involvement in the regulation of food intake and adipogenesis.[5,6] We investigated the expression of PACAP and its three receptors in the adipose tissue of rats fed for eight weeks with high fructose diet (HFD), by quantitative immunohistochemistry and stereology, to determine whether HFD can influence neuropeptide expression in adipose tissue. We showed that PACAP and its receptors are expressed in adipose tissue of control and HFD rats; while PACAP, PAC-1 and VPAC-2 expression is not altered by HFD, VPAC-1 expression is largely inhibited by fructose treatment. Furthermore, HFD rats showed a 30% increased adipose cell number and increased adipocyte volume. These findings show that HFD can alter morpho-functional characteristics of adipose tissue, suggesting that VPAC-1 down regulation is correlated to HFD-induced hypertrophy and hyperplasia of adipose tissue.

References

1. Tomimoto S et al. J Pharmacol Sci 2008;107: 41-8.
2. Akesson L et al. Endocrinology 2003;144:5293-9.
3. Vaudry D et al. Pharmacol Rev 2009;61:283-357.
4. Wei Y and Mojsov S, J Neuroendocrinol 1996;8:811-7.
5. Yu R et al. Acta Physiologica Sinica 2008;60:751-8.
6. Lijnen RH et al. J Obes 2010;2010: pii: 359527, 1-5.

HER-2 OVEREXPRESSION IN UNUSUAL HISTOTYPES OF GASTRIC CARCINOMAS

A. Ieni, G. Giuffrè, V. Barresi, R. Scarfì, R. Caruso, G. Tuccari

Dept. of Human Pathology, Sections of Pathological Anatomy, A.O.U. "Polyclinic G. Martino", University of Messina, Messina, Italy. E-mail: tuccari@unime.it

Background. Recently, HER2 overexpression and/or amplification has been revealed also in gastric cancer with a mean value about 20% of cases, although carcinomas localized at the gastroesophageal junction and the intestinal histotype showed an increased immunopositive percentage up to 32%. In the present study, we have immunohistochemically analyzed the HER2 status in a cohort of gastric adenocarcinomas, pointing out the HER2 positivity in unusual histotypes, such as hepatoid and mitochondrion-rich carcinomas, which represent the opposites findings in relation to prognosis and survival. *Methods.* One-hundred-six formalin-fixed paraffin embedded gastric surgical specimens were obtained from an equal number of patients (M 66 - F 40); mean age 67.8 (range 35-95 yrs); mean follow-up value 40.5 months. Clinico-pathological data concerning site, histotypes according WHO as well as Lauren, grade, nodal status, staging and growth fraction (Ki-67) were also available. HER-2 status (A0485 DAKO, Denmark) has been evaluated by a score: 0 (no staining), 1+ (faint and discontinous staining in < 10% of neoplastic elements), 2+ (light to moderate lateral, basolateral or complete staining in > 10% of neoplastic elements), 3+ (strong, intense lateral, basolateral or complete staining in > 10% of neoplastic elements). All cases considered equivocal (2+) have been furtherly assessed by FISH test (pharmDx DAKO). Statistical analysis was performed by Chi-square test. *Results.* 57 cases showed intestinal histotype, 9 of which were mitochondrion-rich tumors, 26 were diffuse and 23 gastric carcinomas were mixed-type, 11 of which were hepatoid. HER2 overexpression showed a progressive increase moving from diffuse (4.17%) to mitochondrion-rich (11,1%), intestinal (26.4%) and hepatoid (36.3%). Moreover, HER2 overexpression was significantly related to grade, stage, high Ki-67 value and survival.

IMMUNOHISTOCHEMICAL STUDY OF HER-2 OVEREXPRESSION IN INDOLENT GASTRIC CARCINOMAS

G. Giuffrè, A. Ieni, V. Barresi, A. Simone, G. Branca, R. Caruso, G. Tuccari

Dept. of Human Pathology, Sections of Pathological Anatomy, A.O.U. "Polyclinic G. Martino", University of Messina, Italy. E-mail: giuffre@unime.it

Background. HER2 overexpression and/or amplification has been reported in advanced gastric cancer with a mean value about 20% of cases, but no informations are available in early gastric carcinomas. In the present study, we have immunohistochemically analyzed the HER2 status in a cohort of indolent gastric adenocarcinomas, pointing out the HER2 positivity in mitochondrion-rich carcinomas, which have been considered a low-grade malignancy with an excellent prognosis. In parallel, we have also investigated HER status in early gastric carcinomas, in which the neoplasm affects only the mucosa and/or the submucosa, irrespectively of their lymph node colonization. *Methods.* 10% neutral formalin-fixed paraffin embedded thirty-three surgical specimens of indolent gastric carcinoma (mainly early) were obtained from an equal number of patients (M 20 - F 13); mean age 67.8 (range 55-80 yrs). Clinicopathological data concerning site, histotypes according WHO as well as Lauren, grade, nodal status, staging and growth fraction (Ki-67) were also available. HER-2 status (A0485 DAKO, Denmark) has been evaluated by a score: 0 (no staining), 1+ (faint and discontinous staining in ≤10% of neoplastic elements), 2+ (light to moderate lateral, basolateral or complete staining in ≥10% of neoplastic elements), 3+ (strong, intense lateral, basolateral or complete staining in > 10% of neoplastic elements). All cases considered equivocal (2+) have been furtherly assessed by FISH test (pharmDx DAKO). Statistical analysis was performed by Chi-square test. *Results.* 24 cases showed intestinal histotype, 9 of which were mitochondrion-rich carcinomas, while 9 were diffuse type. HER2 overexpression was found in 11.1% of mitochondrion-rich and in 12.5% intestinal histotypes, without any significant difference. Additionally, no relationships emerged between HER2 overexpression and clinico-pathologic parameters.

EXPRESSION PROFILES OF MATRIX METALLOPROTEINASES 15 AND 19 IN HUMAN COLORECTAL CARCINOGENESIS

P. Sena,[1] F. Mariani,[2] L. Marzona,[1] L. Roncucci,[2] C. Palumbo[1]

[1]Dept. Biomedical Sciences, Human Morphology Section and [2]Internal Medicine, University Modena-Reggio Emilia, Italy. E-mail: paola.sena@unimore.it

Introduction. The matrix metalloproteinases (MMPs) have been well characterized for their ability to degrade extracellular matrix proteins and thus they have been extensively studied to elucidate their involvement in both tumour development and progression. In the present study the attention were focused on two members of MMP family, i.e. MMP-15 and MMP-19. *Methods.* The expression profile of MMP-15 and 19 were assayed from samples of normal mucosa, microadenomas and cancer using confocal analysis, Western blotting and quantitative reverse transcription polymerase chain reaction (Q-PCR). *Results.* The semiquantitative immunofluorescence analysis showed that MMP-15 expression level increases from normal mucosa to microadenomas, with a reduced level in cancer respect to microadenomas, while MMP-19 staining increases in normal mucosa-microadenoma-carcinoma sequence. Both Q-PCR and Western blot methods correlate with immunofluorescence behaviour of MMP-15 and 19, showing a significantly higher expression of MMP-15 in microadenomas compared to normal mucosa, and indicating an increasingly amount of MMP-19 levels in the progression of colon lesions. *Conclusions.* MMP-15 and 19 appear to be up-regulated during tumorigenesis, with different expression patterns, that are probably due to the different roles played by these two molecules. The literature up to now reported show conflicting data regarding the role of these proteins in tumor progression so that the improved understanding of the biological roles of MMPs in colorectal cancer should lead to a re-evaluation of the use of MMP inhibitors and highlight the importance of integrated translational studies on the MMP expression patterns.

NESTIN EXPRESSION IN HUMAN BREAST CANCER: IMMUNOHISTOCHEMICAL STUDY

F. Piras,[1] S. Lai,[1] M. Piga,[2] M.T. Ionta,[3] F. Atzori,[3] L. Minerba,[4] M.T. Perra,[1] C. Maxia,[1] D. Murtas,[1] P. Demurtas,[1] V. Pusceddu,[3] M. Murru,[3] B. Massidda,[3] P. Sirigu[1]

[1]Dept. of Cytomorphology, University of Cagliari, Monserrato (CA); [2]Dept. of Pathology, SS. Trinità Hospital, Cagliari; [3]Dept. of Medical Oncology, and [4]Dept. of Public Health, University of Cagliari, Italy. E-mail: s_lai@unica.it

Breast carcinoma is the most common malignancy and is the second leading cause of cancer death in women. It is a heterogeneous disease embracing several different phenotypes with consistently different biological characteristics. Nestin, an intermediate filament protein, was originally identified as a marker of neuroepithelial stem/progenitor cells in the brain. Recently, it was observed that nestin is preferentially expressed in basal/myoepithelial cells of the normal mammary gland and that this protein may be used as a myoepithelial marker. Nestin was also later found in a cell population with cancer stem cells (CSC) characteristics in many tumors, such as breast cancer. However, the clinical and prognostic implications of nestin as a marker for breast cancer are still unclear. A consecutive series of 53 patients with T4 breast carcinoma was enrolled between 1992 and 2001 in Sardinia, and observed up for a median of 125 months. Patients were assessed by physical examination and mammography, confirmed via core needle biopsy. All patients completed a treatment plan including primary chemotherapy, surgery, radiation therapy, adjuvant chemotherapy, and hormone therapy. Archival paraffin-embedded tissue sections were used for immunohistochemistry (IHC) analyses, in order to assess alterations in expression levels of nestin. The Kaplan-Meier and Cox regression methods will be used for survival assessment and statistical analysis. The results will be discussed.

THE EPIDEMIOLOGY OF GENITAL HUMAN PAPILLOMAVIRUS IN WOMEN IN BENIN, WEST AFRICA

F. Piras,[1] M. Piga,[2] A. De Montis,[3] A.R.F. Zannou,[4] L. Minerba,[5] M.T. Perra,[1] D. Murtas,[1] S. Lai,[1] C. Maxia,[1] P. Sirigu[1]

[1]Dept. of Cytomorphology, University of Cagliari, Monserrato (CA), Italy; [2]Dept. of Pathology, SS. Trinità Hospital, Cagliari, Italy; [3]Research Laboratories, bcs Biotech S.p.A., Cagliari, Italy; [4]Ministère de la Santé, Cotonou, Bénin; [5]Dept. of Public Health, University of Cagliari, Italy. E-mail: fpiras@unica.it

Cervical cancer ranks as the 1st frequent cancer among women in Benin. The major cause of cervical cancer now recognized is HPV persistent infection. In Benin there is a lack of screening programs for prevention of cervical cancer and little

information exists regarding HPV genotype distribution. Cervical cells from 725 women were examined for the presence of viral DNA by means of a PCR multiplex-based assay, and of abnormal cytology by Papanicolaou method. The association between HPV status and Pap test reports was evaluated. Socio-demographic and reproductive characteristics were also related. A total of 18 different HPV types were identified, with a prevalence of 33.2% overall, and 52% and 26.7% among women with and without cervical lesions, respectively. Multiple HPV infections were observed in 40.2% of HPV-infected women. In the HPV-testing group, the odds ratio for the detection of abnormal cytology was 2.98. High risk types were involved in 88% of infections, most notably HPV-59, HPV-35, HPV-16, HPV-18, HPV-58 and HPV-45. In multiple infections of women with cytological abnormalities HPV-45 predominated. This study provides the first estimates of the HPV prevalence and type-specific distribution among women from Benin and demonstrates that the epidemiology of HPV infection in Benin is different from that of other world regions. Specific area vaccinations may be needed to eradicate cervical cancer and the other HPV-related diseases.

EXPRESSION OF PI-PLC β2 IN BREAST CANCER PROGRESSION FROM *IN SITU* TO INVASIVE

V. Bertagnolo, F. Brugnoli, M. Palomba, S. Grassilli, E. Nika, S. Capitani

Signal Transduction Unit, Section of Human Anatomy, Dept. of Morphology and Embryology, University of Ferrara, Italy. E-mail: bgv@unife.it

Although the majority of breast cancer deaths occurs as a result of metastasis rather than from the effects of the primary lesion,[1] at present no marker has been clearly associated with the progression of breast lesions from *in situ* to invasive.[2] In breast, as in a wide variety of tissues, phosphoinositide-dependent phospholipases C (PI-PLCs) are involved in malignant transformation. In particular, the expression of the β2 isozyme, almost absent in normal mammary cells, characterizes primary invasive breast tumors, in which it positively correlates with the de-differentiation levels of malignant cells, with biological and clinical-pathological factors currently used to characterize invasive breast carcinomas and with a poor prognosis of patients.[3,4] To assess the involvement of PI-PLC β2 in the switchover of breast tumors from *in situ* to invasive, a retrospective analysis of PI-PLC β2 expression in sample tissues from *in situ* and invasive carcinomas was performed and the levels of PI-PLC β2 were correlated with the most common histological and biological prognostic and predictive parameters for breast tumors. The results indicated that the expression of PI-PLC β2 characterizes the transition of mammary cells from normal to tumoral but not the evolution of breast cancer from *in situ* to invasive. On the other hand, we have found that different PI-PLC β2 levels are expressed by adjacent cells of *in situ* carcinomas. Since most breast cancers evolve from precursors which gradually change over time,[5] populations expressing different PI-PLC β2 levels may represent different stages of tumor progression from *in situ* to invasive.

References

1. Morrison et al., Breast Cancer Res 2008;10210.
2. Sommers et al., Breast Cancer Res Tr 1994;31:325-35.
3. Bertagnolo et al., Int J Oncol 2006;28:863-72.
4. Bertagnolo et al., Carcinogenesis 2007;28:1638-45.
5. Allred et al., Clin Cancer Res 2008;14:370-8.

SEM ANALYSIS OF PARTICLES AND IONS RELEASE ON PERIPROSTHETIC MEMBRANE

M. Sabbatini,[1] G. Gatti,[2] L. Marchese,[2] M. Cannas[1]

[1]Dept. Clinical and Experimental Medicine; [2]Dept. Advanced Science and Technologies, University of Piemonte Orientale; Italy. E-mail: maurizio.sabbatini@med.unipmn.it

The arthroplasty clinical failure will be taken after the loss of bone that occurs through the activity of resorption stimulated by inflammatory cells in response to the presence of debris. The type of prosthesis and bearing surface used have a significant impact on the potential for wear debris development and following osteolysis. We investigate the distribution and relationship of wear debris and metal ions. To assess the influence of immune response aseptic and septic arthroplastic loosening were studied. 28 specimens of bone perimplant interface membrane were taken at the time of first revision surgery for septic (cemented: n=6; cementless: n=6) and aseptic (cemented: n=8; cementless: n=8) loosening. Age of patient: 68±6 years. Duration of implant: 8±2 years. The cementless prosthesis had Ti_6Al_4V treated cups, coupled to titanium alloy SCL stems; the cemented prosthesis, had (UHMWPE) cemented cups, coupled with (PMMA) cemented Co-Cr alloy stems. Each specimen was fixed in 10% buffered formalin, and processed for paraffin embedded. Organic and metallic nanoparticles were counting and analysed under SEM. Ion distribution and quantification were evaluated by X-ray spettroscopic analysis. Organic or metal wear particles did not showed different distribution between several membranes analysed in number and average size, although the absence of a more detailed analysis about the different composition of particles may hide any differences. The distribution of ions showed an high presence of different ions released from metal alloy forming prosthesis. These finding may better explain the occurring of inflammatory phenomena, and toxicity events that are recognize to bring directly to implant loss.

CHARACTERISATION OF CIRCULATING ENDOTHELIAL CELLS (CECS) IN PERIPHERAL BLOOD

P. Lanuti,[1,2,3] L. Raskit,[1,2] L. Pierdomenico,[1,2,3] M. Marchisio,[1,2,3] S. Miscia[1,2,3]

[1]Section of Human Morphology, Department of Medicine and Aging Science (D.M.S.I.), University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy, [2]Center for Aging Sciences (Ce.S.I.), "Università G.d'Annunzio" Foundation, Chieti, Italy, [3]StemTeCh Group, Chieti, Italy

Circulating endothelial cells (CECs) are very rare events in the peripheral blood that have a high diagnostic value in different diseases, such as cancer, cardiovascular diseases and diabetes. 8-colour flow cytometric panel was optimized for the evaluation of CECs in peripheral blood. So far, no single marker can characterise CECs, therefore, a combination of at least two markers is necessary. The combination of three different endothelial cell markers in this panel enables a reliable analysis of CECs. The use of a DNA stain, a live/dead marker and the leucocyte antigen CD45 excludes unwanted cells, microparticles and platelets from the analysis. In addition, an activation marker (CD106) and a precursor marker (CD117) were included in the panel for further characterization of CECs. The absolute numbers of CECs per ml blood were calculated by acquiring an additional tube with TruCount beads and whole blood stained with Syto16.

DECELLULARIZATION OF RAT AND HUMAN OMENTUM TO DEVELOP NOVEL SCAFFOLDS FOR REGENERATIVE MEDICINE

A. Porzionato,[1] M.M. Sfriso,[1] V. Macchi,[1] C. Stecco,[1] G. Sarasin,[1] L. Lancerotto,[2] V. Vindigni,[2] R. De Caro[1]

[1]Dept. of Human Anatomy and Physiology, University of Padua; [2]Clinic of Plastic Surgery, University of Padua, Italy. E-mail: rdecaro@unipd.it

Background. The omentum may be an interesting source of decellularized scaffold maintaining a complex physiological 3D structure, completed with vessels, to be recellularized by autologous cells and it would have numerous applications in the field of reconstructive surgery. *Methods.* Adult rat and human omenta were treated with an adapted decellularization protocol involving freeze-thawing cycles, enzymatic digestions with trypsin, deoxyribonuclease, lipase and ribonuclease, and lipids extraction to yield a collagenous natural matrix, i.e., decellularized adipose tissue (DAT). The scaffolds obtained were studied with histological (haematoxylin-eosin, azan-Mallory, Van Gieson, Oil Red and Sudan) and immunohistochemical (anti-CD31, -laminin, -collagen IV) stainings to highlight the absence of cells and lipids, and the persistence of the frames of the 3D structure and vascular network. *Results.* Histological stainings confirmed the effectiveness of the decellularization protocol, resulting in a cell-free scaffold with no residual cells and lipids. Azan Mallory and Van Gieson stainings and immunostaining for laminin and collagen type IV identified a large amount of collagen and elastic fibers, organized in a quite complex three-dimensional network that still preserved vascular structures. The volume of samples was quite preserved during the decellularizing passages. *Conclusions.* The fat-rich and well vascularized omental adipose tissue may be decellularized to realize complex tridimensional scaffolds preserving the architecture of tissue and suitable for recellularization without any rejection. Further analysis will have to verify the possibility of recolonization of the scaffold by autologous cells such as Adipose Derived Stem Cells (ADSC) *in vitro* and then *in vivo* after reimplantation, as already known for homologus skin implants in regenerative processes.

MAO IN THE OPTIC NERVE OF YOUNG AND OLD RAT

C. Cavallotti,[1] N. Pescosolido,[2] F. Mignini[3]

[1]Dept. of Human Anatomy, University "La Sapienza" Rome, Italy; [2]Dept. of Geriatric Sciences, Section of Ophthalmology, University "La Sapienza" Rome; [3]School of Pharmacy, Experimental Medicine Unit, University of Camerino, Italy. E-mail: cavallotti@uniroma1.it

Age-related changes of the mono-amino oxidase (MAO), evaluated by enzymatic staining, quantitative analysis of images, biochemical assay and statistical analysis of data were studied in the optic nerve samples of young (3-month-old) and aged (26-month-old) male Sprague-Dawley rats. The histochemical findings were compared with biochemical results. Staining of the MAO and biochemical assay of the same enzyme were performed according to the method suggested by Uchida and Koelle. The reaction was evaluated by incubating sections (for histochemical analysis) or homogenates of the samples (for biochemical assay) in a medium either with or without substrate or/and with or without dye. The reaction was considered to be positive when nitroblue tetrazolium (dye) precipitates were present. In this case the granules appear blue on slides and black on B/W photographs. The quantitative analysis of images (QAI) was performed on slides using a Quantimet 500 Leica® apparatus , as reported with further details in the Handbook of Methods Leica. A statistical analysis was performed both for histochemical and biochemical results Considering the results of our experimental procedures, we can affirm that MAO levels in the optic nerve of rats increase with age. The increase of enzymatic staining shows a close correlation with the progression of age and/or senile alterations. The possibility that age-related changes in MAO levels may be due to impaired energy production mechanisms or if these changes represent the consequence of reduced energetic needs is discussed.

EFFECTS OF OP/NP MIXTURE ON HYPOTHALAMUS-PITUITARY-ADRENAL GLAND AXIS OF THE LIZARD *PODARCIS SICULA*

A. Sellitti,[1] M. De Falco,[1] R. Sciarrillo,[2] S. Valiante,[1] A. Capaldo,[1] V. Laforgia[1]

[1]Dept. of Biological Sciences, Section of Evolutionary and Comparative Biology, University of Naples Federico II; [2]Dept. of Biological and Environmental Sciences, University of Sannio, Benevento; Italy. E-mail: madefalco@unina.it

Endocrine disrupting chemicals (EDCs) may interfere with the functioning of endocrine system of several animal species including human. They act by genomic mechanisms with agonist or antagonist effects on estrogen or androgen receptors. EDCs act by non-genomic mechanisms too, interfering with steroid hormone synthesis and/or metabolism. Among EDCs, two alkylphenols (APs), octylphenol (OP) and nonylphenol (NP), are persistent compounds and ubiquist pollutants of aquatic environment. The aim of this study was to evaluate the *in vivo* effects of NP and OP mixture on hypothalamus-pituitary-adrenal gland axis of bioindicator lizard *Podarcis sicula* by biochemical and histochemical approaches. Reptiles are suitable as contaminant biomonitors due to their persistence in a variety of habitats, wide geographic distribution, longevity, site fidelity and to their ability to bioaccumulate. We demonstrated that prolonged exposure to NP and OP mixture induced a significant increase of CRF, ACTH and corticosterone plasma levels in a dose-dependent manner. NP/OP mixture also induced a high increase of adrenaline plasma levels and a contemporary decrease of noradrenaline plasma levels. Moreover, we observed an intense hypertrophy of steroidogenic cells together with a great increase of vasculogenesis of the whole gland. We also found an increase of adrenaline cell number and a complete degranulation of chromaffin cells. This study showed that NP/OP mixture caused a continuous and strong stimulation of hypothalamus-pituitary-adrenal gland axis with a lack of negative feedback.

DOPAMINERGIC SYSTEM EXPRESSION IN RAT FACIAL NUCLEUS AND HIPPOCAMPUS AFTER TRIMETHYLTIN ADMINISTRATION

F. Mignini,[1] C. Nasuti,[1] C. Capezzone,[1] F. Giovannetti,[2] G. Iannetti,[2] L. Fumagalli[3]

[1]School of Pharmacy, Experimental Medicine Unit, University of Camerino; [2]Dept. of Maxillo-Facial Surgery and [3]Dept. of Human Anatomy, University Sapienza of Rome, Italy. E- mail: fiorenzo.mignini@unicam.it

Trimethyltin (TMT) is known to cause neuronal degeneration in the central nervous system with neurotoxicant effects especially marked in the hippocampus. TMT is considered a useful tool to obtain an experimental model of neurodegeneration.[1,2] Despite many studies are published, the mechanisms by which TMT induces neurodegeneration are still not understood, in particular there are poor literature on the interaction of xenobiotic with dopaminergic system. In the present work, we investigate animal behaviour in association with the immuno-

hystochemical expression of the dopaminergic system (D1- and D2-like receptors and dopamine membrane trasporters DAT and vesicular monoamine transporter VMAT-1 and -2) and cells viability (N-NEU) in the hippocampus and facial nucleus regions following TMT intraperitoneal administration in rat. The animal behaviour, at 21th day of study, shows a significant reduction of spatial reference memory in a Morris water maze task according with the reduction (70% Vs control) of dopaminergic system expression obtained in hyppocampus, despite the cell viability is maintained at about 50%. In the facial nucleus, at 21 days post-treatment, a different reduction of dopamine receptors and trasporters (30% against 60%) was observed. The N-NEU reduction was 40%. These results suggest that the toxic interaction of TMT with the dopaminergic system in rat hippocampus may be responsible for learning and memory deficits. Data obtained in facial nucleus demonstrate different sensitivity of dopamine receptors and dopamine transporters to xenobiotic.

(Supported by PRIN 2008 - prot. 20089MANHH_002)

References

1. Pompili E et al., J Histochem Cytochem, 2011, 59: 302-11.
2. Geloso MC et al. Neurochem, 2011

NEUROPEPTIDE EXPRESSION AND CD3+ T RECRUITMENT IN THE RAT FACIAL NUCLEUS FOLLOWING PERIPHERAL NERVE TRANSECTION.

F. Mignini,[1] F. Giovannetti,[2] C. Nasuti,[1] M. Cocchioni,[1] G. Iannetti,[2] L. Fumagalli[3]

[1]School of Pharmacy, Experimental Medicine Unit, University of Camerino; [2]Dept. of Maxillo-Facial Surgery, and [3]Dept. of Human Anatomy, University Sapienza of Rome, Italy. E-mail: fiorenzo.mignini@unicam.it

Axonal injury in peripheral nerve can have a strong impact on the neurons and the surrounding non-neuronal cells.[1] Peripheral nerve injury (PNI) induces changes in gene expression of a variety of neuroactive substances which include neuro-immuno-modulatory molecules and regeneration associated genes.[2] Some neuropetides exhibit several actions on neurons, glia and inflammatory cells *in vitro* that could be beneficial in nerve injury.[3] Several results have been obtained in designed studies on axotomy model, but the literature is lacking about the vasoactive intestinal peptide (VIP) and substance P (SP) expression in facial nucleus (FN) region after PNI. Here we present some immunohistochemical data concerning VIP and SP, and CD3+T-cells recruitment in the rat facial nucleus (FN) at 5, 14 and 21 days after PNI. VIP and SP expression were strongly induced in FN (with a peack at 5 days), whereas the CD3+ pan-T cells peacked at 14 days post axotomy. VIP expression in FN may be dependent on the extent of nerve damage and might modify the immune response after injury; SP could play a critical role in immune response and the decline of SP expression could suggest a beginning of nerve regeneration. In conclusion the present data suggest that i) FN neurons are under neuropeptidergic influence, and ii) T cells may be involved in preventing initial neurodegeneration or neuronal death.

(Supported by PRIN 2008 - prot. 20089MANHH_002)

References

1. Madura T et al. Brain Res. Mol. Brain Res 2004;24:76
2. Kreutzberg GW Arzneimittelforschung 1995;45:357.
3. Waschek JA Dev. Neurosci 2002;24:14.

EXPRESSION OF DOPAMINE RECEPTORS IN THE CORPUS LUTEUM OF PSEUDOPREGNANT DOES

F. Mignini,[1] M. Zerani,[2] M. Capacchietti,[1] C. Boiti,[3] C. Cavallotti,[4] F. Parillo[2]

[1]School of Pharmacy and [2]School of Veterinary Medicine Science, University of Camerino, Italy; [3]Faculty of Veterinary, University of Perugia, Italy; [4]Faculty of Medicine and Surgery, Sapienza University, Rome, Italy. E-mail: fiorenzo.mignini@unicam.it

Dopamine is a neurotransmitter that modulates the functions of various systems: central, autonomic and peripheral nervous, cardiovascular, and renal.[1] Up to date, five specific subtypes of G protein-coupled dopamine receptors (DR) have been found, which are divided into two superfamilies: DRD1-like (D1 and D5, positively coupled with adenylate cyclase) and DRD2-like (D2, D3, and D4, negatively coupled or uncoupled with adenylate cyclase).[2] Recently, it was suggested that dopamine could affect the ovary function after its conversion into noradrenaline,[3] but a direct modulatory action and/or different activity of dopamine on ovarian tissues cannot be excluded. In the present study, the expression of DRD1- and DRD2-like receptors was evaluated in corpora lutea (CL) of rabbits collected at early- (day 4), mid- (9) and late- (13) luteal stages of pseudopregnancy,[4] by immunohistochemical and PCR techniques. Immunohistochemistry evidenced the presence of DRD1and DRD3 in the luteal cells at all stages considered, whereas DRD2, DRD4, and DRD5 were weakly immunoexpressed. The PCR data confirmed the gene expression for DRD1, DRD2 and DRD3 in the CL. These preliminary data suggest the hypothesis that the lifespan of rabbit CL could be also modulated by dopamine receptors.

References

1. Goldeberg LI Pharmacol Rev 1972;24;1.
2. Cavallotti C et al. Heart Vessels 2010;25;432.
3. Kings et al. Domest Anim Endocrinol 2005;28;405.
4. Zerani M et al. J Endocrinol 2010;207:289.

THE ROLE OF THE PROTEASE-ACTIVATED RECEPTOR-1 IN RAT MICROGLIA EXPOSED TO TRIMETHYLTIN

E. Pompili,[1] C. Fabrizi,[1] E. De Santis,[1] S.L. Nori,[2] B. Panetta,[1] M.C. Geloso,[3] V. Corvino,[3] F. Michetti,[3] L. Fumagalli[1]

[1]Dept. of Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza University, Rome; [2]Dept. of Pharmaceutical Sciences, University of Salerno; [3]Institute of Anatomy and Cell Biology, Università Cattolica del Sacro Cuore, Rome, Italy. E-mail: elena.desantis@uniroma1.it

Trimethyltin (TMT) is an organotin compound that causes, in the rat brain, severe hippocampal neurodegeneration associated with astrocyte and microglia activation. Previously, we showed that TMT-activated astrocytes expressed the protease-activated receptor-1 (PAR-1),[2,3] the prototypic member of a family of four G-protein-coupled receptors that signal in response to thrombin and other extracellular proteases. In the nervous system PAR-1 has been shown to be involved in several brain pathologies.[4] In the present experiments we extended our investigation to microglial cells. Interestingly, by 7 days following TMT intoxication *in vivo*, confocal immunofluorescence revealed an evident PAR-1-related specific immunoreactivity in OX-42-positive microglial cells of the CA3 and hilus hippocampal regions. Furthermore, when primary rat microglial cells were treated *in vitro* with TMT, a strong upregulation of PAR-1 was observed by immunocytochemistry and Western

blot analysis. Further experiments were also performed to investigate the significance of this PAR-1 up-regulation in microglia after TMT exposure. In particular, we studied the expression of some metalloproteinases (MMPs) in microglial PAR-1 positive cells by triple immunofluorescence experiments.

References

1. Balaban et al. Neuroscience 1988, 26:337-61
2. Pompili et al., Brain Res Mol 2004, 122:93-8.
3. Pompili et al. Int J Mol Med 2006, 18:33-9
4. Sokolova and Reiser Thromb Haemost. 2008, 100:576-81.

HETEROGENEITY OF MOSSY FIBRE TERMINALS IN THE GRANULAR LAYER OF RAT CEREBELLAR CORTEX AS REVEALED BY IMMUNOHISTOCHEMISTRY FOR VGLUT-1, VGLUT-2 AND SNAP-25

V. Benagiano, L. Lorusso, P. Flace, F. Girolamo, A. Rizzi, L. Bosco, G. Ambrosi

Dept. of Human Anatomy and Histology R. Amprino, University of Bari, Bari Italy. E-mail: benagiano@anatomia.uniba.it

The mossy fibres (MF) are the main source of inputs to cerebellar cortex. They originate from neurons localized in brainstem and spinal cord and project to cortex of all cerebellar lobes; an additional *intrinsic* MF also exist in the flocculonodular lobe originating by the unipolar brush neurons. According to classic views, MF end in the granular layer, where they form glutamatergic excitatory synapses on granule dendrites. GABAergic terminals from Golgi, candelabrum and Lugaro neurons modulate these synapses, so constituting synaptic complexes (glomeruli). Inputs from aspartatergic, noradrenergic, cholinergic and serotonergic neurons in the brainstem, vehicled to cerebellar cortex by non-glutamatergic MF, have been also described. In the present study, using techniques of multiple labelling in immunofluorescence, we analyzed the distribution in the granular layer of immunoreactivity for VGluT-1 and VGluT-2, glutamate transporters which specifically characterize glutamatergic terminals, and SNAP-25, a protein localized on the membrane of synaptic terminals involved in the regulation of exocytosis of neurotransmitter-containing vesicles. The results indicated that immunoreactivity for VGluT-1, VGluT-2 and SNAP-25 is present in terminal-like structures lying in large numbers in the interstitial spaces among granule bodies. Multiple labelling experiments revealed:

- co-localization of VGluT-1 with VGluT-2, of VGluT-1 or VGluT-2 with SNAP-25;
- elements positive for VGluT-1 but negative for VGluT-2 and vice versa, elements positive for VGluT-1 or VGluT-2 but negative for SNAP-25;
- elements expressing positivity only for SNAP-25.

The present study indicates that terminals of MF largely express VGluT-1 and VGluT-2, markers of glutamatergic neurotransmission, and SNAP-25, which is associated with mechanisms of glutamatergic neurotransmission. However, subpopulation of MF terminals which do not co-localize VGluT-1 and VGluT-2 or do not express SNAP-25 also exist. The terminals negative for VGluT-1 and VGluT-2 and positive for SNAP-25 could be represented by the non-glutamatergic contingent of MF.

α-TOCOPHEROL ROLE IN DENDRITIC SPINE REMODELLING OF ADULT RAT DENTATE GYRUS

P. Ferri, F. Buccella, P. Ambrogini, M. Betti, T. Cecchini, S. Ciaroni

Dept. of Earth, Life and Environmental Sciences, University of Urbino "Carlo Bo". E-mail: paola.ferri@uniurb.it

Our previous studies demonstrated that α-tocopherol induces an increase of granule PSA-NCAM positive dendrite length and synaptic profile density in the dentate gyrus molecular layer of adult rat, suggesting that synaptic reorganization processes occur. In this study, the expressions of spinophilin, a postsynaptic spine glycoprotein, and of MAP2, a cytoskeletal protein used as a dendrite remodelling marker, were evaluated after α-tocopherol administration. Western blotting analysis showed a significant increase of spinophilin in hippocampus of α-tocopherol treated rats with respect to controls. This result was confirmed by the densitometric analysis of spinophilin immunoreactivity in the dentate gyrus. Indeed, the spinophilin densitometric analysis demonstrated a significant increase both in the granule cell layer and in the molecular layer, where it was due to an increase in each band of the molecular layer. Western blotting analysis did not show differences in MAP2 expression between treated and control rats, according to densitometric analysis results, which showed no significant differences both in the granule cell and in the molecular layer. Given the relationship between MAP2 and spinophilin proteins and synaptic connection plasticity, our results, demonstrating a spinophilin increase but an unchanged MAP2 expression, show that α-tocopherol may be able to play a role in structural changes related not to dendrite cytoskeleton reorganization but to plasticity events affecting postsynaptic spines only. These results suggest an α-tocopherol ability in promoting dendritic spine formation and/or growth, according to previous data demonstrating a synaptogenesis increase after α-tocopherol administration.

EPENDYMOGLIA SPINAL CORD MODIFICATION IN A MODEL OF NEURONAL PLASTICITY

I. Ferrandino,[2] R. Imperatore,[1,3] G. Zampi,[1,2] R. Favorito,[1,2] A. Di Nunzio,[1,2] L. Cristino[1]

[1]Inst. of Cybernetics, CNR of Pozzuoli, Italy; [2]Dept. of Biological Sciences, University Federico II, Naples; [3]Dept. of Neurological Sciences, University of Verona, Italy. E-mail: l.cristino@cib.na.cnr.it

Unlike adult mammalians and birds, in which the predominant glial elements are astrocytes, the ependymoglia is main in reptiles. In the present study we investigate the modification of the spinal cord ependymoglia in a model of neuronal plasticity as the regeneration of tail in lizard *Gekko gecko*. The caudotomy induces the growth of regenerating blastema and its differentiation with complete replacement of the amputated segment. A rudimental spinal cord re-grows into regenerated tail. It consists of an ependymal cell tube originating from the ependyma lining the central canal of the rostral spinal cord to the amputation plane, through to create a cellular scaffold that guides and supports the re-growing central axons. The aim of the present study is to investigate the morphological changes of glial elements in the tail spinal cord of lizard with intact tail compared to the relative region committed to innervate the regrowing tail at 5, and 15 days after caudotomy, and the complete regenerated tail. Single and multiple immunohistochemistry were performed for identification of glial fibrillary acid protein (GFAP) coupled to nuclear neuronal marker (NeuN). Intensely labelled GFAP-immunoreactive elements were found during spinal cord regeneration compared to intact tail. Many

of these cells were tanycytes since they belong to subependymal layer and to gray matter of ventro and dorso-lateral part of central lumen. Each tanycyte abuts the lumen and extends a single, large processes into gray matter. The ependymal origin and radial arrangement of tanycytes were evident as they originated perpendicularly from the ependymal lumen and coursed parallel each other. These features suggest a changing of role for these cells, very likely involved in the supportive scaffold of the re-growing axons.

NEUROPROTECTIVE ROLE OF ENDOCANNABINOIDS IN A MODEL OF AXONAL DAMAGE AND REGENERATION

R. Imperatore,[1,3] I. Ferrandino,[2] R. Favorito,[1,2] G. Zampi,[1,2] A. Di Nunzio,[1,2] L. Cristino[1]

[1]Inst. of Cybernetics, CNR of Pozzuoli, Italy; [2]Dept. of Biological Sciences, University Federico II, Naples, [3]Dept. of Neurological Sciences, University of Verona, Italy. E-mail: l.cristino@cib.na.cnr.it

Endocannabinoids are lipid signaling mediators with neuromodulatory and neuroprotective roles in several types of brain injury. Unlike mammalian, the nervous system of low vertebrates is promptly able to regenerate neurons and spinal nerves after unjury. In the lizards, tail loss transects spinal nerves and the cut axons elongate in the regrowing tail, providing a natural paradigm of robust regenerative response of injured spinal motoneurons. On this basis we have investigated the possible involvement of the endocannabinoid system in the survival of motoneurons committed to axonal elongation. By our previous studies, a typical chromatolytic reaction to axotomy appears by 10-14 days of caudotomy in the so called "reactive" which is followed by "regenerative phase" up to the complete regrowing of tail. In the present work, we compared the endocannabinoid system of regenerating motoneurons, at 10 days (reactive phase) and at 4 months after caudotomy (regenerative phase), to those of intact motoneurons. Single and multiple immunohistochemistry were performed for CB1 and CB2 receptors, 2-AG endocannabinoid synthesizing (DAGL alpha) and degrading (MAGL) enzymes, glutamate and GABA vescicular transmitter transportes (VGluT1 and VGAT, respectively), glial marker GFAP and the activated microglial marker Iba-1. Our data suggest a general decrease of endocannabinoid system activity during the reactive phase. In the regenerative phase we observed a strong CB1-ir on both glutamatergic and GABAergic axon terminals surrounding motoneurons expressing DAGL-α. CB-2-ir and Iba-1-ir came back to normal levels. These results support a neuroprotective role of 2-AG via inhibition of glutamate-mediated neuronal excitotoxicity.

HYPEROXIA-INDUCED CHANGES IN MORPHOMETRIC PARAMETERS OF THE SUBVENTRICULAR ZONE AND HIPPOCAMPAL DENTATE GYRUS

A. Porzionato,[1] V. Macchi,[1] C. Stecco,[1] M. Baraldi,[2] A. Rambaldo,[1] G. Sarasin,[1] M.M. Sfriso,[1] P. Zaramella,[2] L. Chiandetti,[2] R. De Caro[1]

[1]Dept. of Human Anatomy and Physiology; [2]Department of Pediatrics, University of Padua, Padua, Italy. E-mail: rdecaro@unipd.it

In literature many works address the effects of hypoxia exposure on postnatal neurogenesis but few data are available about hyperoxia effects, although high oxygen concentrations are frequently used for ventilation of premature newborns. Thus, the aim of the present study was to compare with controls the morphometrical parameters of the main neurogenic sites in newborn Sprague-Dawley rats exposed to 60% or 95% oxygen for the first 14 postnatal days. Six rats were studied for each of the three groups. The unbiased quantitative method of the optical disector was applied to analyze neuronal densities, nuclear volumes, and total neuron numbers of the subventricular zone and hippocampal dentate gyrus. Apoptosis was also studied by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL) method. The study was performed in accordance with the Italian Public Health Office regulations and under appropriate ethical committee approval. The subventricular zone of newborn rats exposed to 95% hyperoxia showed statistically significant higher volume (mean value ± coefficient of variation: 0.40±0.20 mm^3) than subventricular zones of rats raised in normoxia (0.20±0.11 mm^3) or 60% hyperoxia (0.26±0.18 mm^3). Total neuron number was also higher in 95% hyperoxia. Conversely, the dentate gyrus of the normoxic rats showed lower volume (0.65±0.11 mm^3) than both the hyperoxic groups (60% hyperoxia: 0.39±0.14 mm^3; 95% hyperoxia: 0.36±0.16 mm^3). Hyperoxia-exposed rats showed higher apoptotic indexes than controls in both suprapyramidal and infrapyramidal bands of the dentate gyrus. Our findings indicate that hyperoxia exposure in the first postnatal period may differentially affect neurogenic areas inducing neuronal degeneration, mainly apparent in the dentate gyrus, and a compensatory neurogenic response, mainly evident at the level of the subventricular zone with an increase in volume and total neuron number.

A NEW LIPOPHILIC MOLECULAR COMBINATION CODRUG-1 EXERTS NEUROPROTECTIVE ACTIVITY IN β AMYLOID (1-40) INFUSED ALZHEIMER'S DISEASE RAT MODEL

S. Zara,[1] A. Di Stefano,[1] M. Rapino,[2] D. Bosco,[2] V. di Giacomo,[3] P. Sozio,[1] A. Abbadessa,[1] A. Cataldi[3]

[1]Drug Science Dept, [2]CNR Molecular Genetics Inst,Chieti, [3]Medicine and Ageing Science Dept, Human Anatomy Section, Faculty of Pharmacy, University "G. d'Annunzio" Chieti-Pescara. E-mail: s.zara@unich.it

Alzheimer's disease is a neurodegenerative pathology due to the presence of brain β amyloid plaques and associated to loss of memory, speech and learning.[1] Since current evidences show the non-steroidal anti-inflammatory drug, ibuprofen, to have a protective effect against the development of the disease, substantially delaying its onset and (R)-α-lipoic acid to have an antioxidant ameliorating effect on its progression, a new lipophilic molecular combination, codrug 1, obtained by joining these two compounds has been synthesized[2] and our aim has been to investigate its possible therapeutical effects on the molecular events at the basis of behavioural and morphological modifications occurring in Aβ infused rat brains. Codrug 1 seems to protect the subject against memory performance impairment and behavioural detriment, induced by administration of Aβ. Such evidences are supported by morphological and biochemical findings showing Aβ to determine cell disorganization,increased number of β amyloid plaques and capillary vessels dilatation in parallel to increased specific and total NOS[3] activity and to apoptosis occurrence, partly prevented by ibuprofen, more broadly by codrug 1. Such results suggest codrug 1 as a useful tool to protect the brain against cognitive and behavioural dysfunction, by reducing β amyloid plaques formation and by inhibiting NOS signalling and apoptosis occurrence.

References

1. Zettenberg H et al. Exp Gerontol 2010;45:23-9.
2. Di Stefano et al. Int J Immunopathol Pharmacol 2010;23:589-99.
3. Malinski T. J Alzheimer Dis 2007;11:207-18.

PDE5 INHIBITION COUNTERACTS β-ADRENERGIC STIMULATION OF CARDIAC HYPERTROPHYC INDUCTION

A. Di Grazia,[1] M. Cornacchione,[1] A. Zevini,[1]
V. De Arcangelis,[1] M. Pellegrini,[1] A. Isidori,[2] L. Monaco,[3]
F. Naro[1]

[1]D.A.H.F.M.O. section of histology and medical embryology, [2]Dept. of Medical Physiopathology, [3]Dept. of Physiology and Pharmacology, University of "La Sapienza", Roma, Italy. E-mail: adigrazia2000@yahoo.it

The β-adrenoceptors play important roles in cardiovascular function regulation mediated by the sympathetic nervous system. Indeed, sustained β-adrenergic stimulations promote cardiac hypertrophy.[1] The β-adrenoceptor agonist Isoproterenol (ISO) is a widely used agent to induce cardiac hyperthrophy.[1] Recently, an anti-hypertrophic role of phosphodiesterase5 (PDE5) has been reported in mice hearts where hypertrophy was mechanically induced.[1] Here we show that PDE5 plays a role on ventricular cardiomyocyte hypertrophy induced by β-adrenergic stimulation. Using a three-dimensional model of cardiac tissue and isolated cardiomyocytes obtained from neonatal mice,[3] we observed that ISO induced cardiac hypertrophy markers and this effect was counteracted by Sildenafil, a specific inhibitor of PDE5. Moreover, morphometric analyses on isolated cardiomyocytes cultured for 48h with ISO, showed a 2-fold increase of cellular size but Sildenafil addition prevented this increase. Furthermore, after ISO treatment RNA and protein levels of PDE5 were increased on cardiomyocytes and this event did not occur in myocytes treated with both ISO and Sildenafil. Finally, PDE5 levels increased specifically after β-adrenergic but not following α-adrenergic stimulations that induce hypertrophy. In conclusion PDE5 inhibition could be used to prevent hypertrophy induced by β-agonists and have therapeutic potential.

References

1. Oleg et al., Heart Fail Rev 2007, 12:66-86
2. Takimoto et al., Nature Med 2005, 11:214-222
3. Xiang et al., PNAS 2005, 102: 909-914

Index of authors