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133 **Study of different cell kinetic markers (DNA content, PCNA, p53, apoptosis, karyotype) in colorectal adenomas and adenocarcinomas**

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It is well documented that most colorectal adenocarcinomas arise in pre-existing adenomas. Colon adenomas have therefore an unpredictable potential to become cancers, but the detection of these changes is difficult by both histologic and clinical evaluation.

The aims of this study were 1) to examine in large samples of colon adenomas (55 cases) and adenocarcinomas (74 cases) different biological markers of cell proliferation by combined immunohistochemistry for PCNA index, apoptosis and p53 evaluation, densitometry for DNA content determination and interphase cytogenetics by fluorescence in situ hybridization (FISH) for numerical chromosome alterations detection; 2) to determine whath markers can indicate the risk of malignant transformation in colon adenomas.

A considerable variability of results was observed in both pathologic groups. Overall, 25% of the adenomas, independently from histological grading, showed nearly the same biological pattern of the larger part (89%) of adenocarcinomas, with a DNA-aneuploid content, p53 over-expression, trisomies and tetrasomies of chromosome 11, low percentage of apoptotic nuclei and very high PCNA index.

It is concluded that the joined presence of some biomarkers (additional chromosomes 11, high PCNA and DNA indices, p53 overexpression, low apoptotic index) can allow to identification of the more aggressive colon adenomas, able to cancer transformation.

134 **A mechanism for Rb/p130-mediated transcription repression involving the recruitment of the CtBP corepressor.**

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Previous work has demonstrated the critical role for transcription repression in quiescent cells through the action of E2F-Rb or E2F-p130 complexes. Recent studies have shown that at least one mechanism for this repression involves the recruitment of histone deacetylase. Nevertheless, these studies also suggest that other events likely contribute to E2F/Rb-mediated repression. Using a yeast two hybrid screen to identify proteins that specifically interact with the Rb-related p130 protein, we demonstrate that p130, as well as Rb, interacts with a protein known as CtIP. This interaction is dependent on p130 sequence that is important for repression activity as well as an LXCXE sequence, a motif previously shown to mediate interactions of viral proteins with Rb, within CtIP. CtIP interacts with CtBP, a protein named for its ability to interact with the C terminal sequences of adenovirus E1A. Recent work has demonstrated that the Drosophila homolog of CtBP is a transcriptional co-repressor for Hairy, Knirps, and Snail. We now show that both CtIP and CtBP can efficiently repress transcription when recruited to a promoter by the Gal4 DNA binding domain, thereby identifying them as co-repressor proteins. Moreover, the full repression activity of CtIP requires a PLDLS domain that is also necessary for the interaction with CtBP. We propose that E2F-mediated repression can occur in at least two complementary fashions, either through the recruitment of a histone deactylase or through the recruitment of the CtIP/CtBP co-repressor complex. The elucidation of a mechanism for histone deacetylase-independent CtBP mediated repression is currently in progress. (ARM is supported by DAMD17-98-1-8074)

135 **THE P53/MDM2 AUTOREGULATORY LOOP IS NOT CONSTITUTIVELY OPERATIONAL *IN VIVO*.**

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MDM2 and p53 participate in an autoregulatory loop in which p53 can induce expression of its own inhibitor. The p53/MDM2 autoregulatory loop has been elucidated within cultured cells; however, this autoregulatory pathway has not been demonstrated to be operational within intact tissues. We have examined the role of p53 in regulating *mdm2* expression *in vivo* in order to test the hypothesis that the p53/MDM2 autoregulatory loop is the mechanism by which low levels of p53 are maintained. We demonstrate that basal expression of *mdm2 in vivo* is p53-independent. In contrast, *mdm2* transcription is induced in a p53-dependent manner following γ-irradiation. These results indicate that p53 regulates *mdm2* expression *in vivo* following genotoxic stress, but that a factor other than p53 must regulate basal expression of *mdm2*. Together, results obtained with tissues, as well as with mouse embryo fibroblasts (MEFs), indicate that the p53/MDM2 autoregulatory loop is not constitutively operational but can become activated in response to stimuli. Therefore, we are investigating whether *mdm2* expression is regulated by p53 under all conditions of stress that activate the checkpoint function of p53 including oncogene expression, ribonucleotide depletion, and aberrant VDJ recombination.

The ability of MDM2 to bind to p53 and stimulate its degradation as well as physically block p53's ability to transactivate gene expression in cultured cells suggests that MDM2 is a potentially important regulator of p53 function *in vivo*. The negative regulation of p53 by MDM2 may contribute to the mechanism by which low levels of p53 are maintained within a tissue. Alternatively, MDM2 may be required to inhibit p53 function only under conditions of stress when the p53/MDM2 autoregulatory loop has been activated. To distinguish between these possibilities, we are creating a mouse strain in which *mdm2* can be conditionally inactivated. Through gene targeting of embryonic stem (ES) cells we have introduced *loxP* sites into intronic regions of the *mdm2* allele and are currently using these cells to generate mice. Once obtained, these mice will provide the tools necessary for the analysis of the regulation of p53 function by MDM2 *in vivo*. The expression levels of p53 target genes, apoptosis, and radiation sensitivity in *mdm2*-null tissues will be compared to identical tissues expressing wild-type *mdm2*. In this manner, the extent to which MDM2 contributes to the regulation of p53 function both prior to and following genotoxic stress will be elucidated.

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Nuclear Export of Adenomatous Polyposis Coli Protein is Required for Regulation of Nuclear β-Catenin

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Mutational inactivation of the adenomatous polyposis coli (*APC*) tumor suppressor gene is an initiating step in the development of colon carcinomas. Here we demonstrate that APC protein contains two functional leucine-rich nuclear export signals (NESs), each able to induce the nuclear export of a fused carrier protein. Both APC NESs were independently able to bind the Crm1 nuclear export factor and substitute for the HIV-1 Rev NES in mediating nuclear mRNA export. Treatment of cells with the Crm1-specific nuclear export inhibitor leptomycin B resulted in the relocalization of endogenous APC from the cytoplasm to the nucleus. Furthermore, although expression of wild-type APC protein reduced both nuclear β-catenin protein levels and β-catenin/LEF-1-dependent transactivation, neither was down-regulated by APC protein with mutationally inactivated NESs.

Together, these data demonstrate that APC is a nucleocytoplasmic shuttle protein whose predominantly cytoplasmic localization requires NES function. Moreover, these data indicate that nuclear export of APC is critical for down-regulation of nuclear β-catenin, APC's oncoprotein target. The ability of APC to enter the nucleus and target nuclear β-catenin for cytoplasmic degradation suggests a novel mechanism by which APC can reverse the Wnt-dependent nuclear localization of β-catenin and consequently affect β-catenin/LEF-1-dependent transcription of target genes.