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DUSP6/MKP3 - a phosphatase between the MAP ERK and mTOR pathways. Regulation of its expression in tumoral cell lines

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Introduction: We had previously shown that the dual specificity phosphatase DUSP6/MKP-3 was phosphorylated and degraded upon growth factor stimulation, in a MEK-dependent manner (Marchetti et al, 2005). Here we investigated the role of the PI3K/mTOR pathway in the phosphorylation and degradation of DUSP6, induced by serum growth factor.

Materials and methods: CCL39, LS174 and A375 tetracyclin-inducible cell lines were used for the study of the phosphorylation and degradation of DUSP6 upon stimulation with serum and specific agonists for the mTOR pathway such as amino-acids or insulin/IGF. In addition, we used specific pharmacological inhibitors for the PI3 Kinase and mTOR pathway. With different mutant constructions of DUSP6, we identify the residues phosphorylated by the mTOR pathway.

Results: upon serum stimulation, DUSP6 is degradated and undergoes a phosphorylation that is evidenced by an electrophoretic shift. This shift is partially blocked by the PI3K and mTOR inhibitors. Moreover, specific agonists for the PI3K/mTOR pathways were also able to induce phosphorylation and degradation of DUSP6. However, a basal activity of MEK was required for the mTOR pathway-mediated phosphorylation to occur. Mutagenesis studies identified serine 159 within DUSP6 as the target of the mTOR pathway. In order to understand the differences of expression of DUSP6 in tumours, we study now the role of the MAP kinase ERK and PI3K/mTOR pathways in the regulation of the expression of DUSP6 at the RNA and protein level.

Conclusion: the ERK phosphatase DUSP6 constitute a novel branch-point of the cross-talk between two major signalling pathways implicated in cell growth in physiological and tumoral processes, the MEK/ERK pathway and the PI3K/mTOR pathway.

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Modulation of insulin receptor substrate-1 expression is related to differentiation in human colorectal epithelium and colorectal cancer

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The goal of our study is to test whether insulin receptor substrate 1 (IRS1) plays a role in the differentiation of human colorectal epithelia and whether IRS1 expression and functions are involved in colorectal cancer development and progression. Caco2 and HT29 colorectal cancer cell lines were used as models of colorectal epithelial differentiation. IRS1 gene and protein expression was assessed by Real Time-PCR and western blotting analysis and correlated with proliferating or differentiated status of epithelial cells. IRS1 distribution in colorectal crypts was studied by immuno-histochemistry in 24 non-neoplastic human colorectal mucosa specimens.

The IRS1 gene and protein expression was characterized in the CaCo2 differentiation model, which recapitulates colorectal enterocytic differentiation. Data show that IRS1 gene and protein expression increases during the 14 days differentiation time course. Insulin receptor β (Insulin Rβ) subunit protein is weakly and highly expressed in proliferating and differentiated enterocytes respectively. Insulin-like growth factor I receptor (IGF-IR β) subunit protein is highly and weakly expressed in proliferating and differentiated enterocytes respectively. These results suggest that the IRS1 protein could mediate the IGF-IR pathway in proliferating enterocytes and the IR pathway in differentiated enterocytes. IRS1 immunohisto-chemistry of non-neoplastic human colorectal mucosa shows that the protein is moderately expressed in crypt epithelia, with maximum expression in enterocytes at the upper third of the crypt and luminal surface. IRS1 gene and protein expression was also characterized in pre-confluent, confluent and post-confluent HT29 cell lines. Transmission electron microscopy of HT29 cells demonstrates gradual differentiation. Data show that IRS1 gene and protein levels decreased during the 14 days time course and were highly expressed in proliferating HT29 cells and weakly expressed in more differentiated HT29 cells. Insulin Rβ and IGF-IR β subunit protein were modulated during the HT29 cells time course showing increasing and decreasing expression respectively. A primary CRC tissue microarray containing 190 CRC cases was analyzed for IRS1 protein expression. This showed heterogeneous IRS1 expression in colorectal cancer. Association studies with clinicopathologic features are in progress. These results show that IRS1 gene and protein expression is modulated during in vitro and in vivo normal colorectal epithelial and colorectal cancer differentiation, together with the Insulin R and IGF-IR β subunits.

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Signal-dependent control of autophagy and cell death in colorectal cancer cell: the role of the p38 pathway

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Autophagy is a vacuolar process leading to the degradation of long-lived proteins and cytoplasmic organelles in eukaryotes. This process has an important role in normal and cancer cells during adaptation to changing environmental conditions, cellular and tissue remodeling, and cell death. To date, several signaling cascades have been described to regulate autophagy in a cell type-specific and signal-dependent manner.

In an effort to evaluate the impact of p38 signaling on colorectal cancer cell fate, we inhibited p38alfa kinase function by pharmacological blockade or genetic inactivation in several colorectal cancer cell lines and in animal cancer models. Deficiency of p38alfa activity induces a perturbation in the expression profile of a subset of genes that ultimately lead to cell cycle arrest, autophagy and cell death in a cell type-specific fashion. In these cells, a complex network of intracellular kinase cascades controls autophagy and survival since the effect of p38alfa blockade is differentially affected by the pharmacological inhibition of MEK1, PI3K class I and III, and JNK.

We propose that p38alfa can be involved in the management of cellular stress and/or in the regulation of essential metabolic cascades in colorectal cancer cells. Initially, the autophagic response to p38alfa blockade represents a survival pathway, but prolonged inactivation of the kinase leads to cell death. Inhibition of p38alfa-dependent autophagy breaks this process and triggers an apoptotic response. In accordance with this hypothesis, early re-activation of p38alfa induces a significant time-dependent reduction in the autophagic process with a slow re-entry into the cell cycle. Worthy of note, the relationship between metabolic stress, autophagy and cell death in tumorigenesis has been described in epithelial cancer cells both in vitro and in vivo.

Collectively, our results suggest an opportunity for exploiting the pharmacological manipulation of the p38alfa pathway in the treatment of colorectal cancer. Given the number of drugs, currently available or under development, that target the p38 pathway, it stands to reason that elucidating the molecular mechanisms that link p38 and autophagy might have an impact on the clinical translation of these drugs.

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MUC1 targeting tissue-specific expression in breast cancer cultures

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Background: Promoter region of carcinoma associated mucine-like glycoprotein (MUC1) known as DF3 promoter was cloned from human breast carcinoma T47D with high expression of MUC1 confirmed previously. MUC1 promoter was cloned in non-viral vector pDsRed2-C1 instead of CMV standard eucariotic promoter. Transfection efficiency of pDsRed2-C1-MUC1 and pDsRed2-C1 vectors was compared in specific and non-specific cell cultures.

Methods: MUC1 promoter region -686 +31 was amplified from a DNA isolate of T47D using two-round nested PCR method with the following primers: "-686 AseI" 5' - AAATTAATGGACCCTAGG GTTCATCGGAG - 3' and "+94" 5' - TGTGAGGAGCAGCAGCAGG - 3'; "-686 AseI" and "+31 NheI" 5' - AAGCTAGCGATTCAGGCAGGCGCTGGCT - 3'. The PCR product was cloned into pTZ57R/T (PCR products cloning kit, Fermentas). Homology of promoter region was confirmed by sequence data using standard M13 primers. The obtained region was cloned into pDsRed2-C1. Homology was confirmed by sequence data using the following primers: "-565-582" 5' - GGAGGAGGAAGAGGTAGG - 3', "-122-139" 5' - ACCCTGAAACCCACAGTC - 3'. MUC1 antigen expression in cell cultures was studied with flow cytometry (FACSCalibour, BD), transfection was performed using GeneJammer (Stratagen). Transfection efficiency and specifity of expression were evaluated with fluorescent and confocal microscopy (Leica DMIRE SP2).

Results: MUC1-inducible FP expression is breast cancer-specific and detectable after 36-48 hours in contrast to 24 hours for CMV promoter.