Alterations in the Wnt pathway play a major role in colorectal cancers (CRCs) with high (MSI-H) or low microsatellite instability (MSS/MSI-L). However, the differential impact of the Wnt pathway components on these tumours is poorly understood. In order to clarify this effect, we analyzed by oligonucleotides microarrays the expression profile of 113 genes related to the Wnt pathway in 44 tumours classified by their MSI status. These results were validated by Real Time Quantitative PCR. With this technique we confirmed significant differential expression values for DVL2, KREMEN2, PPP2R1B, FBXW4, CSNK1D and TLE3. Transcriptional expression for all of these genes was higher in MSI-H tumours, as compared with MSS/MSI-L group. MSI-H colorectal cancers showed expression profiles nearly to the values detected in the pool of non-tumoral samples. MSS/MSI-L expression levels significantly diminished in relation to normal samples. Therefore, sporadic CRCs from the mutator phenotype pathway and normal colorectal mucosa displayed similar transcriptional profiles for genes above mentioned. In contrast, CRCs from the supressor pathway showed down regulated transcriptional profiles.

Then, several colorectal cell lines were analyzed by Real Time Quantitative PCR in order to check if these six genes showed the same expression profile that we detected in biopsies. We chose three MSI-H colorectal cell lines, HCT15, HCT 116 and RKO, and two MSS colorectal cell lines, Caco2 and SW 480. Real Time Quantitative PCR results indicated that cell lines HCT15, HCT116 and SW 480 had a similar expression profile as in vivo samples. RKO cell line was similar to HCT15 and HCT116 cell lines concerning to gene expression of the selected genes except for FBXW4 which mRNA levels were similar to SW 480 cell line. Surprisingly, Caco2 cell line showed likely mRNA levels to MSI-H cell lines except for DVL2.

In conclusion, our results suggest that the differential expression of genes that negatively regulate the Wnt pathway in MSI-H or MSS/MSI-L colorectal tumours shed some light on the different clinical behaviour showed by the two groups.

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A constitutional translocation t(1;17)(p36.2;q11.2) in a neuroblastoma patient disrupts the the human NBPF1 and ACCN1 genes

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The human 1p36 region is deleted in many different types of tumors, and so it probably harbors one or more tumor suppressor genes. In a Belgian neuroblastoma patient, a constitutional balanced translocation t(1;17)(p36.2;q11.2) may have led to the development of the tumor by disrupting or activating a gene.

Here, we report the cloning of both translocation breakpoints and the identification of a novel gene that is disrupted by this translocation. This gene, named NBPF1 for Neuroblastoma BreakPoint Family member 1, belongs to a recently described gene family encoding highly similar proteins, the functions of which are unknown. The translocation truncates NBPF1 and gives rise to two chimeric transcripts of NBPF1 sequences fused to sequences derived from chromosome 17. On chromosome 17, the translocation disrupts one of the isoforms of ACCN1, a potential glioma tumor suppressor gene. Expression of the NBPF family in neuroblastoma cell lines is highly variable, but it is decreased in cell lines that have a deletion of chromosome 1p. More importantly, expression profiling of the NBPF1 gene showed that its expression is significantly lower in cell lines with heterozygous NBPF1 loss than in cell lines with a normal 1p chromosome. Additionally, meta-analysis of the expression of NBPF and ACCN1 in neuroblastoma tumors indicates a role for the NBPF genes and for ACCN1 in tumor aggressiveness.

The disruption of both NBPF1 and ACCN1 genes in this neuroblastoma patient indicates that these genes might suppress development of neuroblastoma and possibly other tumor types.

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NHE1 is essential for invadopodial-dependent extracellular acidification and matrix digestion

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Degradation of the stromal extracellular matrix (ECM) is a critical process of tumor cell invasion and requires membrane and released proteases focalized at membrane structures called invadopodia. Invadopodia are very similar in structure and function to osteoclast podosomes responsible for bone degradation. Extracellular acidification is central to podosome action and, by analogy, could also be for invadopodial function. However, nothing is known concerning either the role of extracellular acidification or the mechanisms driving it in tumor cells. We propose that NHE1 is localized at invadopodia and is necessary for the matrix-degrading activity of tumor cells. Experiments were conducted in metastatic breast cancer cells seeded onto 3D lattices of gelatin, collagen or matrigel in which quenched BSA- or collagen-FITC was mixed and invadopodia activity evaluated microscopically. Focal proteolysis produces fluorescence in a black background which is used both to quantitatively measure proteolytic activity levels and in 3D co-localization analysis with NHE1 expression determined in two independent ways: (i) endogenous NHE1 was analyzed with a polyclonal antibody and (ii) in cells transfected with a GFP-NHE1 construct. Immunofluorescence analysis showed that invadopodial-dependent degradation of the ECM is tightly associated with NHE1 expression. Zones of focal ECM digestion had pH values ranging from 6.5 to 7.1 compared to 7.35-7.5 for the extracellular areas next to cells where digestion had not occured. Exposure of tumor cells to low medium pH increased both NHE1 activity and invadopodial-dependent ECM proteolysis with a increase in invadopodial distribution, length and association with NHE1. ECM degradation was inhibited by blocking NHE1 activity with either its specific inhibitor, cariporide, by transfecting cells with a siRNA against NHE1 or by transfecting cells with transport-deficient mutated NHE1 constructs. Further, cariporide dose-response kinetics were similar for the inhibition of both the NHE1 and ECM digestion suggesting that ECM digestion is dependent on NHE1 activity. We conclude that NHE1 and its associated extracellular acidification are localized to cancer cell invadopodia and are necessary for invadopodial ECM digestion.

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NHERF1 programs invasive and metastatic behaviours in breast tumor cells

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We have reported that elevated NHERF1 expression in human breast cancer is associated with poor prognosis probably through the ability of NHERF1 over-expression to enhance cell invasion through its PDZ2 domain. However, others have observed that NHERF1 over-expression reduces breast cancer cell proliferation and tumor size. To gain insights into the apparently controversial role of NHERF1 in tumor progression, we stably transfected a metastatic breast cell line, MDA-MB-231, with the pcDNA 3.1/Higro empty vector, with wildtype (wt) NHERF1 or with NHERF1 mutated in either the PDZ1 (HRF1) or PDZ2 (HRF2) domains and tested these clones for their ability to affect growth and metastasis both in vitro and in vivo.We show that anchorage-independent growth and in vivo tumor formation are reduced upon wt-NHERF1 and HRF2-NHERF1 over-expression and increased by HRF1-NHERF1 over-expression with respect to pcDNA 3.1. Experiments conducted in 3D matrigel lattices followed by 3D microscopical optical sectioning of the invadopodia marker, cortactin, demonstrate that NHERF1 induces both invadopodium formation and invadopodial dependent extracellular matrix (ECM)-degrading activity through its PDZ2 domain. Finally, BALB/c-nu/nu mice subjected to intracardiac injection of NHERF1-expressing cells demonstrate that expression of HRF1-NHERF1 correlates with increased visceral metastases and HRF2-NHERF1 increased metastasis to bone.We propose that NHERF1 can differently reprogram the tumor progression phenotype by specific loss of function of its PDZ domains. In support of this hypothesis, we show that up-regulation of NHERF1 in breast cancer cells can either suppress tumor growth principally via its PDZ1 domain and promote the acquisition of an in vivo invasive phenotype by inducing invadopodia formation via its PDZ2 domain.

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Human colon cancer stem cells gene profiling

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Background: Recently, several studies have reported that only a minority of cancer cells are responsible for tumor initiation, maintenance and spreading. These "tumor-initiating cells" that display the properties of stem cells (i.e, self-renewal and multilineage differentiation potential) have been termed "cancer stem cells" (CSC). To date, distinct subpopulations of CSC, identified by the expression of specific cell surface markers have been

isolated in tumors of various origins. Particularly, the expression of CD133 antigen has been proposed as the hallmark of CSC in colon cancer. The aim of our study was first to assess the presence of a CD133+ cell fraction in samples of colon cancer and hepatic metastasis of colon cancer. In a second time, we attempted to characterize the CD133+ CSC population, using the human colonic adenocarcinoma caco-2cell line.Material & methods: Tumor and normal tissue samples were obtained from consenting patients who underwent tumor or metastasis surgical resection. Samples were mechanically fragmented and digested by 0,2% collagenase I. Cell suspensions were filtered and characterized by flow cytometry, using CD133, CD45 (hematopoietic cell contamination) and CD31(endothelial cell contamination) antibodies. Further cell characterization was done with caco-2 cells in order to work with a more homogeneous cellular model. Briefly, CD133+ cells were isolated from caco-2 cells by FACS sorter. The tumor-initiating potential of the isolated CD133+ cells versus the remaining CD133- fraction was assessed by soft-agar assay for colony formation. Furthermore, molecular profiling of both subpopulation was performed by the means of microarray analysis.Results: CD133+/CD45-/CD31- fractions (purity >95%) resulted to be significantly higher in both primitive colon cancer (median: 6%) and metatasis (median: 14,7%) compared with their normal tissue counterparts (respectively 0.2% and 0,45%). In vitro assays showed that only CD133+ caco-2 cells were able to generate clones on soft agar. Affymetrix molecular profiling of CD133+ versus CD133- caco-2 cells showed a significant number of genes differentially expressed between both subpopulations. By functional categorization, we identified various targets associated to stemness maintenance overexpressed in the CD133+ cell fraction. Conclusion: Our data demonstrate the presence of a significant CD133+ cell fraction in colon primitive tumor and metastasis. Using the caco-2 cellular model, we showed that only the CD133+ cells exert a tumor-initiating potential in vitro. Furthermore, microarray analysis revealed a unique molecular profile of the CD133+ cell fraction that could be useful to define new“targeted” therapeutic strategies to cure colon cancer.

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TP53INP1 expression inhibits pancreatic tumor cell migration in vitro and in vivo

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Pancreatic cancer progression is regulated by both genetic and epigenetic processes. Characterization of genes that exhibit altered expression during pancreatic cancer development and mechanisms of their function may help to design new therapeutic strategies. TP53INP1 (Tumor Protein 53 Induced Nuclear Protein 1) is a p53-dependent stress response gene whose expression is lost during pancreatic tumorigenesis. We have recently shown that TP53INP1 can induce cell cycle arrest and apoptosis and function as a tumor suppressor in pancreatic cancer cells. The aim of the present work is to characterize a potential role for TP53INP1 in pancreatic cancer cell migration.

The human pancreatic adenocarcinoma cell line MiaPaCa2, which does not express TP53INP1, was modified to express it inducibly. Here we show that upon injecting into pancreas of Nude mice, induction of TP53INP1 expression inhibited migration of these cells into peritoneal cavity in vivo compared to uninduced, TP53INP1 negative cells. In vitro studies also showed a similar difference between TP53INP1 expressing and non-expressing MiaPaCa2 cells when grown on Fibronectin-1 coated dishes, but not on uncoated plastic dishes. In fact, TP53INP1 positive cells formed fewer clones than non-expressing cells in clonogenic assays carried out on fibronectin-1 coated dishes. Furthermore, TP53INP1 expressing cells exhibited reduced migration in Boyden chamber assays with Fibronectin-1 as a chemoattractant. These results show that TP53INP1 regulates pancreatic cancer cell growth as well as their migration on Fibronectin. In order to study the molecular mechanisms mediating this function of TP53INP1, gene expression profiles of TP53INP1 expressing and non-expressing MiaPaCa2 tumors were analysed by DNA microarray. Interestingly, two extracellular matrix-associated genes were found among the most differentially expressed molecules; (a) Fibronectin-1, ove-rexpressed in TP53INP1 expressing tumors and (b) SPARC (Secreted Protein Acidic and Rich in Cysteine) which was downregulated in presence of TP53INP1. These results were confirmed by qPCR. SPARC is a protein that is involved in cell-matrix interactions, cell migration and tumor-stromal interactions. Finally, we show that TP53INP1 expressing MiaPaCa2 cells exhibited transcriptional downregulation of SPARC only when they were grown on fibronectin-1 coated dishes.

In conclusion, the present work describes a novel function for TP53INP1 in regulating pancreatic cancer cell migration and modulation of extracellular matrix-associated genes Fibronectin-1 and SPARC.

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Glucose microenvironment regulates trancript levels of HIF1alpha and of its targets in angiogenesis and glycolysis through the RNA-binding protein PAIP2

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Oxygen microenvironment regulates angiogenesis and energy metabolism through the pVHL/HIF system. Under low oxygen, the Hypoxia-Inducible Factor HIFalpha subunit protein is stabilized and protected from tumor suppressor protein pVHL ubiquitination. However, angiogenesis is probably more crucial to provide glucose to ischemic or to cancer cells than to provide oxygen. In fact, low glucose increases VEGF level and, surprisingly, high glucose also increases it. To investigate the role of HIF system in glucose signalling to VEGF, we incubated isolated renal tubule suspensions under physiological conditions of oxygenation for 4 hours. The results showed that HIF-1alpha and -2alpha mRNA levels, but not HIF-3alpha level, were markedly enhanced below 3mM glucose and also above 7mM glucose. The HIF-inducible mRNAs VEGFA, TIE-2/TRK, FLK1/VEGFR2, aldolase A and GLUT1/SLC2A1 changed in parallel with HIF1alpha messenger whereas targets of CHREBP, the high glucose sensitive transcription factor, L-pyruvate kinase/PKLR and acetyl-CoA carboxylase transcripts, were not significantly altered. HIF1alpha protein was hardly detectable under either condition. Antioxidants did not reverse glucose effects. Half-life measurements showed that low and high glucose effects on mRNAs were essentially mediated by stabilization, in correlation with increased levels of Poly-A binding-Interacting Protein 2 (PAIP2), a mRNA-stabilizing protein that is thought to favor IRES-dependent translation. Therefore the intriguing mechanism that, from two opposing signals, ends in a single response, is to be searched upstream of this protein in the mRNA stabilizing machinery. In conclusion, low glucose and high glucose could potentiate low oxygen effect on HIF protein level. In addition they could be strong signals to activate glycolysis and angiogenesis in a HIF-independent way in the presence of oxygen. The HIF targets recruitment under high glucose could participate in rapid glucose homeostasis by kidney, however during long periods of hyperglycemia an associated angiogenesis and vascular permeabilization could be deleterious and favor microangiopathies and cancer development.

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Rod specific phosphodiesterase 6 regulates human melanoma cell cytoskeleton

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The second messenger guanosine 3’5’-cyclic monophosphate (cGMP) regulates cell processes, such as proliferation, survival, contractility and migration. Notably, the levels of cGMP are reduced in most cancer cells compared to normal cells. Previous studies have demonstrated that there are a family of cGMP phosphodiesterase that are responsible for degrading cGMP, but which are restricted to photoreceptor cells. Thus, PDE6A and PDE6B are restricted to rod cells, whereas the related protein PDE5 is ubiquitous. Thus, as expected, we did not find PDE6A to be expressed in normal human melanocytes, but surprisingly it is expressed in melanoma cells. PDE6B is also expressed in melanoma cells, whereas PDE5 is expressed in both melanocytes and melanoma cells. In line with the elevated expression of PDE6 family members, we show that cGMP levels are lower in human melanoma cells than in primary human melanocytes. We designed an siRNA library to all cGMP-phosphodiesterases family members and confirm that PDE6A and PDE6B are key regulators, together with PDE5 of intracellular cGMP metabolism in melanoma cells. We used a panel of cGMP-PDE inhibitors and siRNA probes to examine the role of PDE6 family members in melanoma cell biology. Inhibition of PDE6 demonstrates that this isoform does not play a key role in human melanoma cell proliferation, whereas inhibition of the enzyme by Vardenafil and/or augmentation of intracellular cGM levels with YC-1, a guanylate cyclase activator caused a significant change in cell morphology. These changes correlate with increased phosphorylation of vasodilator-stimulated phosphoprotein (VASP) and of Myosin-Light Chain-2 (MLC-2), two known regulators of actin remodelling and acto-myosin-dependent contractility respectively. Our data therefore provide a link between cGMP metabolism, the cell cytoskeleton and changes in morphology in melanoma cells and we show that these cellular effects appear to be mediated PDE6A and PDE6B, isoforms that were previously thought to be restricted to photoreceptor cells. We are currently investigating the role of PDE6/cGMP signalling in melanoma cell migration and invasiveness as these studies may provide novel therapeutic targets for melanoma treatment in patients.