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