RESULTS: In vivo both CDDO-Me and CDDO-Im inhibit angiogenesis in the matrigel sponge assay and KS-Imm tumor growth. In vitro they are able to prevent endothelial cells tubulogenesis when cultured on matrigel. Moreover, from immunofluorescence experiments we observed that treatment with these triterpenoids prevents NF-kB translocation into the nucleus and thereby the activation of downstream pathways. In HUVECs CDDO-Me can inhibit the activation of erk1/2 pathway after stimulation with VEGF. CDDO-Im mechanism of action is now under study.

CONCLUSIONS: Our data confirm that inflammation, angiogenesis and the microenvironment play an important role in tumor progression. Triterpenoids in our hands target both endothelial and tumor cells. The repression of the NF-kB pathway suggests anti-inflammatory effects that may also have an indirect role in angiogenesis inhibition. CDDO-Me is now assessed in the US phase I trial in humans.

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Autocrine hGH upregulates VEGF-A expression and promotes tumour angiogenesis in mammary carcinoma

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The survival and proliferation of mammalian cells depends on the delivery of nutrients and oxygen in addition to the removal of waste products through blood vessels. In order to supply the body with nutrients, new vessels develop from pre-existing blood vessels through a process known as angiogenesis. While angiogenesis is tightly regulated in normal tissues, this process is often deregulated in cancer and important in neoplastic progression.

Autocrine human growth hormone (hGH) plays a key role in oncogenic transformation and progression of mammary cell carcinoma, both in vitro and in vivo. Autocrine hGH also promotes migration, invasion and epithelio-mesenchymal transition in the mammary carcinoma cell line, MCF-7. Here we describe a role for autocrine hGH in the development of tumour angiogenesis.

Using a previously established model of autocrine hGH expression in the mammary carcinoma cell line MCF-7, we demonstrate that autocrine hGH specifically increases vascular endothelial growth factor-A (VEGF-A) mRNA and protein levels in MCF-7 cells. Autocrine hGH production in human mammary carcinoma cells stimulated human microvascular endothelial cell (HMEC-1) survival, proliferation, migration and invasion in co-culture experiments. Furthermore, hGH expression in mammary carcinoma cells significantly stimulated HMEC-1 tube formation in Matrigel. Xenograft studies in immunosuppressed mice demonstrated that autocrine hGH promotes increased tumoural expression of the angiogenic markers VEGF-A and CD31. Autocrine hGH tumours had a greater average mass (2.6-fold) and increased tumour microvessel density (2.5-fold) as determined by CD31 staining. In addition, autocrine hGH tumours had increased immunohistochemical staining for the lymphangiogenesis markers, Podoplanin (3-fold) and Flt4 (3.1-fold).

Finally, we demonstrate that HMEC-1 express endogenous levels of hGH and VEGF-A transcript and that functional antagonism of either hGH with the hGH receptor antagonist, B2036, and/or VEGF-A with the therapeutic monoclonal antibody, Bevacizumab, reduces HMEC-1 survival, proliferation and decreases VEGF-A mRNA levels. In addition, treatment of HMEC-1 with Bevacizumab and/or B2036 reduces HMEC-1 tube formation in vitro.

These studies demonstrate that autocrine hGH promotes tumour angiogenesis in mammary carcinoma, effects which are mediated in part through increased expression of VEGF-A.

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The tumor suppressor CEACAM1 is a direct transcriptional target of SOX9 in colon epithelium

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Inactivation of the transcription factor SOX9 gene in mouse intestine affects the morphology of the colon epithelium and leads to hyperplasia (Bastide P. J Cell Biol 2007). Furthermore, overexpression of SOX9 in colon carcinoma cells resulted in apoptotic cell death increase (Jay P. Cancer Res. 2005) and cell proliferation decrease. This suggests a central role for SOX9 in the renewal of the colon epithelium. Nevertheless, direct transcriptional targets of SOX9 in this tissue are still unknown. A microarray analysis identified the tumor suppressor CEACAM1 as a possible target gene of SOX9. To study the regulation of CEACAM1 expression, we used the HT29Cl.16E colonic cells modified to express, upon doxycycline treatment, wild-type SOX9 or a SOX9 mutant form that lacks the C-terminal transcription activation domain. When SOX9 expression was induced the CEACAM1 protein content, analyzed by immunoblot, increased. On the contrary, the induction of SOX9 mutant resulted in a small decrease of CEACAM1 due to a dominant negative effect of the SOX9 mutant. CEACAM1 mRNA level, measured by real-time RT-PCR, increased 2.4-fold when SOX9 was induced and decreased 0.75-fold when SOX9 mutant was induced. A SOX9 tagged green fluorescent protein (GFP-SOX9) was transfected in SW480 colonic cells and CEACAM1 expression was monitored by immunofluorescence. As expected, overexpression of GFP-SOX9 resulted in an increase of CEACAM1 staining confirming that SOX9 up-regulates expression of CEACAM1. Moreover, we observed that, in vivo, CEACAM1 expression was reduced in colon of SOX9 deficient mouse suggesting an important role for SOX9 in the transcriptional activation of the CEACAM1 gene. The SOX9 binding sequence in the human CEACAM1 promoter was identified by luciferase reporter assays. This sequence (CTCACTGggcCTTTGTT) in position -1418 to -1402 contains a SOX consensus sequence (A/TA/TCAAA/TG) in sense orientation with two mismatches followed by three nucleotides and a perfect SOX consensus sequence in antisense orientation. Chromatin immunoprecipitation analysis provided additional evidence of the binding of SOX9 to the CEACAM1 promoter. In addition, we have found that histone acyl-transferase p300 acted as a SOX9 co-activator of the CEACAM1 promoter. We conclude that the tumor suppressor CEACAM1 is the first direct target of SOX9 identified in colon epithelium and that CEACAM1 is a good candidate to mediate a part of the anti-proliferating and pro-apoptotic activity of SOX9.

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Fibroblasts nemosis signals for growth arrest and a dendritic cell-like phenotype shift in human leukemia cells

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Interactive paracrine signaling between cancer cells and their activated stroma plays an important role in tumor development. Signals from cancer cells can induce stromal fibroblast hyperproliferation associated with increased cell-cell contacts and nemosis. Fibroblast nemosis is a unique novel type of mesenchymal cell activation that leads to production of a distinct set of signaling molecules: HGF/SF, IL-1b, IL-6, IL-8, IL-11, LIF, GM-CSF and prostaglandins.

Since the growth factors and cytokines produced are associated with differentiation of hematopoietic cells, we evaluated the effect of nemosis on human leukemia cell lines. Analysis of leukemic cells was carried out after coculture with preformed fibroblast spheroids.

Nemotic fibroblasts induced a dramatic growth inhibition of those leukemia cell lines lacking expression of c-Met, whereas growth of c-Met-positive cells was unaffected. Moreover, the responding cells showed increased adherence, motility, and chemotaxis. The cell cycle of the c-Met-negative cell lines stimulated by nemosis was arrested at the G0G1 phase. Since the growth arrest was accompanied by morphological changes such as cell elongation and formation of stellate pseudopodia, cell surface phenotype was further determined by FACS. New populations with enhanced expression of CD11c, CD13, CD45RA, CD54 and CD86 were identified in the nemosis-responsive cells. Our results show that stromal fibroblast nemosis produces signals that not only stimulate cell motility and chemotaxis but also induce differentiation to a dendritic-cell-like phenotype.

We provide here the first evidence that nemosis can produce specific signaling to arrest growth and induce differentiation of human leukemia cells. Differentiation of leukemic cells into dendritic cell lineage may stimulate T-cells and influence responses of the immune system to malignancy.

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Differential transcriptional profile of the Wnt pathway in sporadic colorectal cancers with and without microsatellite instability

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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