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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