significance as it was observed in human brain tumor biopsies of various grade.

Conclusions - Caveolin-1 plays a critical role in the aggressiveness of glioblastoma. Caveolin-1 effects are achieved through $\alpha5\beta1$ integrin. Mediator of caveolin-1 effects, $\alpha5\beta1$ integrin is also a marker for glioma aggressiveness and an efficient target for the treatment of glioma especially the ones exerting the highest aggressive phenotype.Caveolin-1 /$\alpha5\beta1$ integrin are diagnostic and prognostic markers for glioma and might be predictive of the response to future anti-$\alpha5\beta1$ integrin therapies.

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Tumor cell NG2 proteoglycan controls cancer progression through its interaction with host Collagen type VI

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Soft-tissue sarcomas are highly aggressive and heterogenous tumours that remain largely incurable. As for most types of tumours, the presence of metastasis at diagnosis, or the evolving of such lesions with time, catastrophically reduces the probability of survival. Factors predicting the formation of metastasis in soft-tissue sarcoma patients are not known and similarly obscure remains the modes through which metastases form in these individuals. We find that NG2 and its putative ECM ligand collagen type VI (Col VI) are highly upregulated in metastases of soft-tissue sarcoma patients and that highly enhanced expression of NG2 in these lesions adversely correlate with patient survival. Relative expression levels of NG2 on sarcoma cells, as determined by qPCR and immunostaining analyses, define their malignancy degree and subpopulations of immunosorted highly enriched NG2+ cells exhibit a strongly aggressive behaviour. However, growth and dissemination of NG2+ cells is strongly impaired in Col VI knock-out mice suggesting that the NG2-Col VI interplay dictates tumour progression in vivo. Adhesion and migration of sarcoma cells expressing intact or truncated variants of NG2 and confronted with purified Col VI tetramers or Col VI+ and Col VI- native matrices isolated from wild type and Col VI null mice corroborate the importance of NG2 in collagen recognition and allowed the pinpointing of the reciprocal binding domains within the two molecules. Thus we demonstrated that NG2 cell surface proteoglycan represents a novel independent prognostic factor in certain types of soft-tissue sarcomas where its relative expression levels in primitive lesions strongly predict future appearance of metastases. Global gene profiling of NG2+ versus NG2-, siRNA treated, cells reveals that the proteoglycan confers a malignant and potentially metastatic phenotype independently of previously identified metastasis-associated gene signatures. We also identify some of these signalling pathways that are activated upon NG2-collagen type VI interaction and propose that in addition to serving as a prognostic biomarker, the NG2-collagen type VI interplay and its downstream effectors may constitute novel therapeutic targets in soft-tissue sarcomas and other tumours where NG2 is upregulated/de novo expressed. Taken together these findings highlight a crucial role of NG2 and its interaction with Col VI in the regulation of tumour progression and metastasis formation, providing the first molecular explanation for its uniqueness as a prognostic/therapeutic tool in soft-tissue sarcomas

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COX-2 transgenic mice as models for epithelial neoplasms

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Epidemiologic, pharmacologic, clinical, and experimental studies document the importance of prostaglandin (PG) signaling in epithelial cancer development. First of all, enzymes involved in PG biosynthesis, such as cyclooxygenase (COX)-2 and/or membrane prostaglandin E synthase (mPGES)-1, are overexpressed in a wide range of premalignant and malignant epithelial tumors, including those of the skin, breast, esophagus, stomach, colorectum, pancreas, prostate and urinary bladder. On the other hand, 15-hydroxy-prostaglandin dehydrogenase (15-PGDH), which is involved in the degradation pathway of PG including PGE2, thus counteracting the activities of COX-2 and PGES, was found to be down-regulated in human epithelial tumors, indicating a tumor suppressor activity of this enzyme.

Transgenic mouse lines with keratin 5 promoter-driven overexpression of cyclooxygenase (COX)-2 develop spontaneously pre-invasive epithelial neoplasms. These were diagnosed by human pathologists to be early-stage lesions in skin epidermis, prostate, and pancreas. In addition, in urinary bladder transitional cell carcinomas were observed. The pre-invasive neoplasms and carcinomas in COX-2 transgenic mice resemble not only on the histological level but also on molecular level (e. g. COX-2-, Her-2, VEGF expression) defined progression stages of human neoplasms. COXibs, selective inhibitors of COX-2-mediated PG synthesis representing a class of an approved prescription drug in human medicine have been found to suppress the transgene-induced phenotype, indicating the cause-and-effect relationship between aberrant COX-2 overexpression and the development of the neoplasms.

Moreover, the chronic systemic excess of PG induced by transgenic COX-2 overexpression caused severe white adipose tissue wasting in these mice. The molecular mechanism leading to this phenotype may explain cachectic body wasting in human cancer patients.

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Understanding the complex crosstalk between p53 and the estrogen receptors at a polymorphic variant of the VEGF receptor Flt-1 promoter

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Recently we established that a C>T single nucleotide polymorphism (SNP) in the Flt-1 promoter generates a functional half-site p53 response element (RE-T). We also showed that p53 is required but not sufficient for Flt-1 transactivation and that there is cooperative interaction with ligand-bound estrogen receptors (ER) via an ER half-site response element (ERE) located 225nt upstream the p53 RE-T. Disruption of the ERE in a reporter construct containing a 1kb fragment of the Flt-1 promoter resulted in loss of p53 responsiveness in HCT116 (p53 wt, weakly ERbeta positive) and U2OS (p53 wt, negative for ER) cells. Surprisingly, we have now observed that disruption of the ERE has no impact on transactivation in MCF7 cells (p53 wt, ERalpha and ERbeta positive) treated with doxorubicin (doxo) to induce p53. Searches for transcription factor binding sites revealed another putative half-site ERE in the promoter fragment located 145bp downstream the p53 RE. Using site-directed mutagenesis, we showed that while the mutation of this second site has no impact, mutation of both EREs greatly reduced transactivation. Over-expression of ERalpha or ERbeta in HCT116 phenocopied the MCF7 results in terms of the EREs contribution. To induce p53 in MCF7 cells we also used the thymidylate synthase inhibitor 5-FluoroUracil (5FU). Although 5FU was similar to doxo in stabilizing the p53 protein and inducing the p21 target gene, there was minimal transactivation of the Flt1-T construct, suggesting that doxo might have a specific impact on the p53, ER transcriptional cooperation or might enlist additional transcription factors/cofactors that contribute to the activation of the promoter. Using HCT116 cells (p53 wt and p53-null clones), which are heterozygous for the C>T SNP, we are also examining the expression of the endogenous Flt-1 gene, using qPCR. The Flt-1 transcript undergoes alternative splicing resulting in a soluble form of the receptor. These experiments are confirming the p53-dependent regulation of the Flt-1 gene and the different impact of doxo and 5FU. Notably, we are also observing an additional layer of complexity in the regulation of the gene, as the relative abundance of the two splice variants is differentially affected by the doxo treatment. This observation is currently being followed up with the development of assay systems probing stress-dependent stability of the two Flt-1 mRNAs, which have distinct 3'UTRs, as well as relative efficiency of alternative splicing.

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Beta endorphin produced by melanoma cells promotes tumor growth and immune escape

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Beta endorphin (BE) is an endogenous agonist peptide for the mu opioid receptor (MOR); its major role consists in releaving the sensation of pain at proximal nerve endings but can also inhibit immune responses. Interestingly, BE has also been found to be secreted in high amounts by several tumors of neuronal and non-neuronal origin where its role remains unclear. This project intended to investigate if BEsecreted by tmelanoma

cells could play a role in cancer progression. When analysing the expression of BE in 30 human melanoma biopsies obtained from patients, we found a correlation between beta endorphin expression and stage of the malignancy (p < 0, 05). We analysed the potential role of BE in preventing immune response against tumor cells and performed a mice model of cancer progression by subcutaneous injection of melanoma B16 cells to both mu opioid receptor deficient mice (MOR -/-) and their WT counterparts. A profound decrease in tumor growth was observed in MOR -/- mice compared to WT animals (median volume 0,2 cm3 versus 0,8 cm3 at day 15 post injection; p < 0,01). This was paralleled by a significant higher infiltration of CD4+, CD8+, NK and dendritic cells at tumor site of MOR -/- mice determined by flow cytometry and immunohistochemistry. Adoptive transfer experiment with PKH-26-labeled MOR -/- leukocytes in combination with PKH-67-labeled WT leukocytes demonstrated that the higher presence of immune cells was not du to a higher recruitment of cells at tumor sites, but rather to proliferation and activation of leukocytes. NK cell activation was indeed increased by the use of BE-blocking antibody.

These findings demonstrate that endgenous beta endorphin secretion by melanoma cells plays a role in tumor growth and immune escape. Blocking beta endorphin therefore appear to be a promising new attractive therapeutic strategy to limit cancer progression.

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The monocarboxylate transporter 1 (MCT1) and Hypoxia-induced MCT4 are key targets promoting tumor cell survival

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In the hypoxic tumor microenvironment, cancer cells switch their glucose metabolism to glycolysis for their energy demands. This is known to generate a large amount of lactic acid that must be rapidly exported to maintain a permissive intracellular pH, essential in sustaining glycolysis, protein synthesis and cell survival. Cells have evolved several transport systems to extrude lactic acid. A large family of H+-linked MonoCarboxylate Transporters, represented by the ubiquitously expressed MCT1, co-transport H+/lactate- in both directions. Interestingly, MCT4, a member of this family widely expressed in tumor cells, is up-regulated in hypoxia by the transcription factor HIF. In addition, the functional expression of MCT1 and MCT4 in the plasma membrane is finely regulated by specific interaction with the glycoprotein chaperone CD147/Basigin.

Why do tumor cells co-express two lactate transporters? What are their respective functions? Are they both essential for tumor metabolism?

Firstly, we show in Ras-transformed fibroblasts, which express only MCT1, that blockade of this transporter (siRNA or AstraZeneca inhibitor) severely restricts cell growth and survival in vitro in hypoxia and in vivo in xenografted tumors in nude mice. Secondly, we show that ectopic expression of MCT4 in these Ras-transformed fibroblasts bypasses the MCT1 blockade. MCT4 expression restored ATP levels and growth in vitro in hypoxia and in vivo, induced tumors to escape the block in MCT1. Thirdly, we demonstrate that silencing CD147 or MCT4 in several human tumor cell lines grown in hypoxia (breast, prostate, colon, head & neck, melanoma) induces rapid cell death when MCT1 is co-inactivated.

We therefore conclude that MCT1 and MCT4 are two key steps in hypoxic tumor metabolism and as such represent new targets for anticancer therapy.

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Hypoxia-induced BNIP3 reduces proliferation of colon carcinoma cells through downregulation of ERK1/2

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The development of solid tumors is often accompanied by the appearance of hypoxic regions. Tumor cells rapidly respond to this new micro-environment by activating the Hypoxia-induced transcription factor HIF, an acknowledged strong promoter of tumor growth. HIF activates a complex gene expression program ensuring cell survival by inducing glycolysis, angiogenesis, autophagy, migration and invasion but also by controlling proliferation. Amongst these adaptive processes the control of tumor cell proliferation by hypoxia remains unclear and largely uncharacterized at the molecular level. We present here new evidence confirming the negative impact of hypoxia (1% O2) on cell proliferation. We also provide proof that the hypoxia-induced HIF-1a gene known as Bcl2/adenovirus E1B 19kDa-interacting protein 3 (BNIP3) does not induce cell death but mediates the decrease in tumor cell proliferation induced by hypoxia. We demonstrate, in both non-tumoral fibroblast (CCL39) and colon carcinoma (BE) cell lines, that BNIP3 triggers a decrease in the level of phosphorylated ERK leading to a substantial increase in the accumulation of the CDK inhibitor p27 and a delay in S-phase entry. We show that inhibition of ERK activity by treatment with the MEK inhibitor U0126 abolished the hypoxic and HIF-1-induced expression of BNIP3 suggesting the existence of a negative feedback loop regulating BNIP3 expression and cell proliferation in tumors. We propose that hypoxia-induced BNIP3 participates in the general HIF-induced adaptive mechanism leading to tumor cell survival through the attenuation of the rate of cell proliferation but also through the activation of autophagy (see Mazure et al. abstract).

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Caveolin-1, a novel Id1 binding partner, and its role on Id1-induced behavioral change in prostate cancer cells

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Id-1 (Inhibitor of Differentiation/DNA binding-1) is reported to promote cell proliferation, invasion and survival in many types of human cancer cells through multiple signaling pathways. However, how Id1 interacts with these pathways and the immediate downstream effectors of the Id1 protein are not known. In this study, we identified a novel Id-1 interacting protein, caveolin-1 (Cav-1), a cell membrane protein and a positive regulator of cell survival and metastasis in prostate cancer. Using immunoprecipitation method, we found that the helix-loop-helix domain of the Id-1 protein was essential for the physical interaction between Id-1 and Cav-1. We also demonstrated in prostate cancer cells that the physical interaction between Id-1 and Cav-1 played a key role in the Id-1-induced epithelial-mesenchymal transition and cell migration as well as resistance to taxol-induced apoptosis. Our results also revealed that the Id-1-induced Akt activation through promoting the binding activity between Cav-1 and protein phosphatase 2A was responsible for the synergistic effect between these two proteins. Our study demonstrates a novel Id-1 binding partner and suggests a molecular mechanism that mediates the function of Id-1 in promoting prostate cancer cell invasion and survival through activation of the Akt pathway [(HKU7478/03M) to XHW and YCW (HKU7490.03M, 7470/04M, NSFC/RGC N_HKU738/03, HKU Foundation Seed Fund, 03)].

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SEMA3F Semaphorin is involved in tumor angiogenesis

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Class-3 semaphorins (SEMA3s) and VEGF are secreted proteins that bind neuropilin receptors suggesting antagonism, although recent studies argue against binding competition. In addition, SEMA3s regulate integrin activation in the vascular system, as well as in tumor cells.

SEMA3F was identified as a tumor suppressor in lung cancer by chromosome mapping of heterozygous deletions. We and others subsequently demonstrated that SEMA3F blocks in vivo tumorigenesis using different animal models. This was associated with inhibition of integrin-mediated attachment and reduced angiogenesis.

We studied intracellular signaling changes due to overexpression of SEMA3F in H157 lung cancer cells and found lower activity of ILK, ERK1/2, AKT and STAT3. Importantly, we observed downregulation of HIF-1α protein, along with VEGF transcription reduction. In a mouse subcutaneous tumorigenesis model, SEMA3F overexpression had a growth-inhibitory effect that was coupled with reduced vascular support. To further our investigations, we adapted the chick chorioallantoic membrane (CAM) assay to test the angiogenic capacity of lung cancer cells transfected with SEMA3F or VEGF165. Our results confirm the anti-angiogenic activity of SEMA3F but also suggest that VEGF repression at the level of transcription is only partially responsible for the inhibition of angiogenesis. Moreover, VEGF stable transfection in SEMA3F-expressing lung cancer cells did not reverse the inhibitory action of SEMA3F on phospho-ERK1/2.

These data suggest that VEGF antagonism by SEMA3F might not explain all of its anti-tumorigenic properties, and thereby support the development of SEMA3F as a therapeutic agent in cancer.

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The effect of covalent linkage and the number of Arg residues on the in vitro cytostatic effect and cellular uptake of daunomycin-conjugates on HepG2 and HL-60 tumour cells

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Daunomycin (Dau) is an anthracycline derivative widely used in cancer chemotherapy. Dau inhibits the growth and division of the cells mainly by