suppression did not decrease the proliferation rate in vitro,rather a slight increase was observed. However, tumour growth generated from LDH-A deficient clones was significantly reduced. LDH-B was not increased by shRNA interference for LDH-A in a compensatory mode, while Hif1α expression was increased and PHD2 and CA9 expression were significantly decreased in the LDH-A deficient clones.

We show that LDH-A is critical for the growth of colon carcinoma cells in vivo but not in vitro. The LDH-A deficiency seems to induce cellular stress resulting in an increased Hif1α expression and a decreased expression of its regulator, PHD2. A reduced expression of CA9 in those cells may depend on an abrogated lactic acid production. The generation of mouse melanoma (B16F10) and mouse lung carcinoma (Lewis Lung) LDH-A shRNA clones has been successful and the effect in HT-29 cells was reproduced with Lewis lung carcinoma cells but not with B16F10 clones.

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Analysis of TGFBI overexpression and silencing in the proliferation, migration and chemoresistance of NSCLC cells

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Background: TGFBI (transforming growth factor-β, induced protein) is an extracellular matrix protein that has been described to mediate cell adhesion to the extracellular matrix by its interaction with integrins. In spite of recent reports dealing with its expression in tumors, little is know about its role in tumor migration and adhesion. In the present work we aim at studying the role of TGFBI overexpression or silencing on cell adhesion, migration, proliferation and resistance to chemotherapy in NSCLC.

Methods: We analyzed the effects of TGFBI silencing in the NSCLC cell line A549, which expresses high levels of this molecule, and TGFBI overexpression in H1299, that shows low basal TGFBI expression. Cell viability was determined by the incorporation of the vital dye neutral red and apoptosis was measured by PARP degradation. Cell adhesion was measured by the fluorescent labelling of adhered cells while their migration was in Boyden chambers. We have also analyzed TGFBI expression in 22 NSCLC cell lines and in 80 samples derived from NSCLC relative to normal lung tissues.

Results: TGFBI silencing in non metastatic A549 cells increased their proliferation and migration, but decreased extracellular matrix cell adhesion and while recovery of TGFBI expression in H1299 metastatic cancer cells decreased their proliferation and migration and induced H1299 cell adhesion to the extracellular matrix.

We also demonstarte that TGFBI overexpression increases tumour cell sensitivity to chemotherapy whereas loss of TGFBI induced resistance.

Expression studies showed a heterogeneous TGFBI expression in the 22 NSCLC cell lines tested. Besides, we studied the correlation between TGFBI expression, tumor stage and resistance to chemotherapy in 80 NSCLC samples.

Conclusion: Loss of TGFBI is able to increase cell proliferation and migration, and to decrease sensitivity to apoptosis, and points it as a candidate tumor suppressor. The study of TGFBI expression in human lung carcinoma relative to normal lung tissues, and its correlation with several pathological and histological parameters, including tumor stage and chemotherapy response, should be explored as a useful tool in a clinical setting.

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Epidermal growth factor receptor distinguishes between stem and transient amplifying cell fate in squamous cell carcinoma cell line

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Cancer cells are phenotypically and functionally unequal in the tumor mass and in established cultures. This is accounted for by a small subpopulation of cancer cells which have the unique ability of stem cells to generate differentiating progeny while maintaining their own number. Regulation of this dual ability is controlled at the level of asymmetric division by mechanisms that are, as yet, not well defined. Our findings suggest that in the squamous cell carcinoma (SCC) cell line, the fate of cancer cells is linked to the expression level and subcellular distribution of epidermal growth factor receptor (EGFR). Interestingly, though essential for epithelial cell proliferation, differentiation and survival, this factor was not found on the surface of cells that satisfy criteria for stem cells, including asymmetric division, high clonogenic potential, expression of stem cell markers and migration profile. We determined that EGFR can be asymmetrically distributed during cell division and identified several cell cycle, TNF-pathway, survival, mitochondria and self-renewal controlling genes that were differentially regulated in EGFR-negative and EGFR-positive cells and whose expression differed in SCC cells and their normal counterparts. Our data suggest that EGFR might be an important cell fate determinant which switches the stem cell phenotype into transient amplifying during asymmetric division, and that the set of genes associated with this switch is different for normal and cancer stem cells.

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Inhibitory effects of unliganded estrogen receptor alpha on breast cancer cell growth and invasion

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Breast cancer, the most frequent malignancy in western women, is a model of hormone dependent malignancy. While estrogens are mitogenic in breast cancer cells, the presence of estrogen receptor alpha (ERα) indicates a favourable prognosis in breast carcinoma. To improve our understanding of ERα unliganded action, we used mutants deleted in ligand and/or DNA-binding domains. In previous studies, we have shown that unliganded ERα protects against invasion through matrigel via protein-protein interaction in its first zinc finger region. Recently, we demonstrated that expression of ERα mutants also inhibits cell outgrowth in three dimensional matrices as well as tumor formation in nude mice. Using GST-pull down and two-hybrid techniques, we found that ERα, via its amino acids 184-283, interacts with the cyclin-dependent kinase inhibitor $p21^{WAF1}$. The interaction between these proteins is detected in absence of estrogens or in the presence of pure antiestrogen $ICI_{182,780}$, whereas estradiol treatment disrupts the interaction. By cross-linking experiments, a large complex of ~200 kDa containing $p21^{WAF1}$, ERα and both cdk2 and cyclin E was identified. We further demonstrate that ERα expression after gene transfection significantly increases $p21^{WAF1}$, while ERα silencing by RNAi significantly reduces $p21^{WAF1}$. Moreover, the silencing of $p21^{WAF1}$ prevents the ERα –induced growth inhibition. In conclusion, our findings point to an anti-invasive and an antiproliferative function of the unliganded ERα through its physical interaction with $p21^{WAF1}$ that may explain, at least in part, the favourable prognosis associated with ERα-positive breast cancers.

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Caveolin-1 regulates glioblastoma aggressiveness through the control of a5β1 integrin expression and modulates glioblastoma responsiveness to SJ749

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Background - Gliomas are the most common deadly brain tumors. Despite advances in neurosurgery, radiation and medical oncology, the prognosis for patient with glioblastoma did not improve in the last 30 years. A better molecular and biological knowledge of glioma will lead to advances for the management of glioma. Increased expression of caveolin-1 seems to be the norm in glioma. As caveolin-1 plays a checkpoint function in the regulation of processes altered in cancer, we investigated its role in glioblastoma carcinogenesis.

Methods - Caveolin-1 was overexpressed or knocked down in U87MG cells and proliferation, clonogenicity and invasion were examined. PCR Arrays were undertaken to determine pathways altered after caveolin-1 manipulation. The involvement of α5β1 integrins was studied by overexpressing or knocking down α5 in U87MG cells and using an antagonist (SJ749). Finally, the expression levels of both caveolin-1 and α5β1 integrin were analyzed in 24 glioma patient samples and normal brain by qPCR.

Results - The reduction of caveolin-1 levels in U87MG shifted cells towards a more aggressive phenotype (increased proliferative, clonogenic and invasive capacity) as conversely the forced expression of caveolin-1 slowed down proliferation, clonogenicity and invasion. Using PCR array strategies, we showed that only 20% of the genes studied were significantly affected by caveolin-1 modulation. The most exciting finding was that half of them belonged to the integrin family and above all that their expression was always inversely correlated to caveolin-1. Focusing on α5β1 integrin, we showed that caveolin-1 could in fact control α5β1 integrin at the transcription level and consequently alters cell sensitivity to the specific α5β1 integrin antagonist, SJ749. We also report here for the first time that the inverse correlation between caveolin-1 and α5β1 integrin had biological

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