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