to low oxygen tension conditions was achieved through the impairment of ubiquitin-dependent HIF-1α degradation involving the molecular chaperone HSP90 but it was not dependent on the prolyl hydroxylation of HIF-1α protein. Notably, we also showed that bcl-2, HIF-1α and HSP90 proteins form a tri-complex that may contribute to enhancing the stability of the HIF-1α protein in bcl-2 overexpressing clones under hypoxic conditions.
Conclusions: We identified the stabilization of HIF-1α protein as a mechanism through which bcl-2 induces the activation of HIF-1 in hypoxic tumour cells, involving the molecular chaperone HSP90.

456 Identification of oncoantigens associated to breast cancer stem cells for preventive antitumour vaccination

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Background: Characterization of genes differentially expressed during the stages of tumour progression may lead to the identification of "oncoantigens", tumour-associated molecules that play important roles in driving tumour progression and constitute potential targets for preventive antitumour vaccination. Until now, we have identified putative oncoantigens (POAs) as molecules expressed by mammary cells in pre-neoplastic lesions and over-expressed in evident neoplastic lesions. However, many human malignancies, including breast cancer, are organized in a hierarchical network of rare slowly dividing cancer stem cells (CSCs), rapidly dividing amplifying cells and differentiated tumour cells. CSCs constitute the source of the tumour and could be responsible for tumour progression, metastasis, resistance to therapy and recurrence, so preventive vaccination should target them. Thus, analysis of CSCs transcriptional profiling may identify new POAs, more suitable for effective vaccination.
Material and Methods: Mammary tumour specimens were obtained from a cell line derived from BALB-neuT breast carcinomas (Ag12). Cells were plated in differentiative conditions to obtain tumour epithelial cells (e0) or under specific conditions to generate mammospheres (p1), which were then disaggregated and plated to obtain second (p2) and third (p3) passage mammospheres. Expression of CSCs markers on mammospheres was checked by cytofluorimetry. Transcription profiling was performed on e0 and p1−3 using Illumina microarray platform MouseWG-6 v2.0.
Results: Mammospheres generated from Ag12 displayed clonogenicity, self renewal, CSCs markers and ability to differentiate in mammary epithelial cells and maintained the tumourigenic potential. 452 deregulated transcripts were detected in mammospheres using rank product statistics, comparing e0 with p1−3. To detect CSC vaccination targets, a subset of 183 transcripts (POAs) which expression increased from p1 to p3 were selected by K-mean clustering. Vaccination targets for breast cancer prevention were selected ranking the 183 transcripts on the basis of the relation between their expression and survival in 7 public human breast cancer transcription profiles. The actual protein increase of some of these POAs in p1−3 was confirmed in ELISA and cytofluorimetric experiments.
Conclusions: Mammospheres transcription profiling led to the identification of new POAs. Future experiments will validate these POAs in preventive vaccination in BALB-neuT mice.

457 Lysophosphatidic acid induces cell-cell adhesion disassembly and actin cytoskeleton disorganization through an event that requires RhoA-Rock and Src signaling in colon cancer cells

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Background: Lysophosphatidic acid (LPA), an extracellular lipid mediator of multiple cellular responses, acts as a potent stimulator of tumour progression triggering different cell signaling pathways that stimulate cell proliferation, migration and survival in colorectal cancer (CRC). Adherens junctions (AJ) disassembly and actin cytoskeleton alterations are initial events of cancer development; however, the cellular mechanisms underlying these phenomenon remain to be defined. The aim of this study was to examine the cell signaling pathways triggered by LPA to mediate alterations of cell-cell adhesion and actin cytoskeleton reorganization during CRC progression.
Material and Methods: Cell monolayers of Caco-2, a colon adenocarcinoma cell line, were used as CRC model. Cells were serum starved for 24 h and then treated with 10 μM of LPA for 15 to 60 min or pretreated for 1 h with inhibitors of Rho GTPases, Rho-kinase (Rock), PI3K, PKA, EGFR and Src, before LPA treatment. Changes in the location of AJ proteins E-cadherin, b-catenin and p120-catenin were examined by immunofluorescence, and actin cytoskeleton organization by confocal microscopy using rhodamine-phalloidin. RhoA and Rac1 activation was assessed by the pull-down assay, and Src and FAK activation through immunoblotting of the phosphorylated protein forms. Cell migration was analyzed through the wound-healing technique, and cell viability through the crystal violet assay.
Results: LPA treatment induced cell-cell adhesion disassembly, alteration of the actin cytoskeleton organization with stress fibers formation. Pharmacological inhibition of Rho with toxin A from *Clostridium difficile* and Rock with Y-27632 prevented AJ disassembly and actin reorganization caused by LPA treatment. Additionally, Src inhibition with PP2 abrogated p120-catenin redistribution from cell-cell contacts to cytosol induced by LPA. We observed that LPA treatment caused RhoA, Src and FAK activation as evidenced by immunoblotting, however RhoA activation was not prevented by Src inhibition with PP2. Furthermore, by the wound-healing technique we demonstrate that Rho, Rock and Src chemical inhibition also prevented the increase in cell migration LPA-mediated.
Conclusions: Our finding indicates that LPA modulates AJ disassembly, actin disorganization and cell migration through a regulatory cascade that integrates RhoA-Rock and Src-FAK signaling pathways in colon tumour cells.

458 Inhibition of TGFβ2 production in mouse dedifferentiated hepatoma cells leads to decrease of their tumourigenic and metastatic potential

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Background: Cytokines of Transforming Growth Factor (TGF) β family are involved in regulation of cell proliferation, apoptosis, motility and differentiation. Also TGFβ plays dual role in carcinogenesis acting as tumour suppressor or promoter depending on stage of tumour progression and tissue context. Increased levels of TGFβ1 were detected in serum and urine of patients with advanced stages of hepatocellular carcinoma (HCC). While the role of TGFβ1 in hepatocarcinogenesis is actively investigated, the impact of other isoforms in this process is underestimated. Our aim was to investigate the role of TGFβ2 in HCC progression. We have shown that in experimental model of mouse HCC progression highly invasive fast-growing HCC (fgHCC) was characterized with overexpression of TGFβ2 and downregulation of HNF4α, liver enriched transcriptional factor playing a central role in maintenance of hepatocyte morphology and differentiation.
Material and Methods: To inhibit production of TGFβ2 in H33 cells culture obtained from fgHCC we used shRNA technique. The effects of TGFβ2 inactivation in H33 cells in vitro were studied by RT-PCR gene expression analysis, proliferation and cell motility tests. To analyze TGFβ2 effects on tumourigenic and metastatic potential of HCC tumour cells were injected subcutaneously into syngenic recipient mice.
Results: TGFβ2 inactivation in H33 cells induced re-expression of HNF4α and C/EBPα, transcription factor also essential for the maintenance of hepatic differentiation, and alterations in several TGFβ2 responsive genes expression. Inhibition of TGFβ2 in H33 cells led to growth retardation and decrease of cell motility in vitro. After subcutaneous injection into mice H33-siTGFβ2 cells showed delay in tumour formation and decrease of metastatic potential.
Conclusions: TGFβ2-induced activation of TGFβ signaling in HCC cells can contribute to tumour progression increasing tumourigenic and metastatic potential of tumour cells. It can be explained by involvement of TGFβ signaling in regulation of such key properties as proliferation, cell motility and differentiation, probably due to repression of HNF4α and C/EBPα.
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459 The FGF-2 binding domain of thrombospondin-1: functional characterization and exploitation to design antiangiogenic compounds

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New blood vessels formed by angiogenesis supply oxygen and nutrients to solid tumours and provide a gateway for metastatic cells to enter the bloodstream and disseminate to distant organs. Thrombospondin-1 (TSP-1), an endogenous inhibitor of angiogenesis, restrains angiogenesis through different mechanisms, including the direct binding to and sequestration of angiogenic factors, in particular fibroblast growth factor-2 (FGF-2). TSP-1 binds FGF-2 through a site located in its type III repeats domain.
We hypothesized that this domain might serve as a template for the development of inhibitors of angiogenesis. Using a peptide array approach, we identified a FGF-2 binding sequence in the type III repeats of TSP-1.