Results: Totally, 75 (55.6%) samples showed overexpression of the HMGA2 protein. Dividing into histological subtypes, 88.9% of squamous cell carcinomas expressed high levels of HMGA2, while the high expression percentage among adenocarcinomas was 45.6%. For the other histological entities combined, 38.7% showed overexpression. The expression levels were not correlated to overall survival in our study. Progression free survival analyses are ongoing.
Conclusion: In this study, we found a strikingly high percentage of high expression of the HMGA2 protein among squamous cell carcinomas. The expression levels showed no effect on overall survival.

444 Inhibition of Stearoyl-CoA Desaturase induces cell death and activation of AMPK pathway in cancer cells

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Cancer cells exhibit altered glycolysis and lipogenesis metabolisms. Indeed, de novo synthesis of saturated (SFA) and monounsatuarated fatty acids (MUFA) is largely increased in cancer cells. The increase of MUFA, is correlated with a higher level of Stearoyl-CoA desaturase (SCD) activity in variety of cancer tumour. SCD is an endoplasmic reticulum enzyme that introduces a double bond between carbons 9 and 10 of several saturated fatty acids such as stearic acid (converted into oleic acid). De novo MUFA production seems to be required for sustaining proliferation and survival of cancer cells. In contrast, down-regulation of Scd1 leads to proliferation arrest and/or cell death with reduction of lipogenesis witch induces activation of the AMPK pathway, the cellular energy sensor. Its activation has been recently discovered to be involved in cell growth arrest and cell death.
In the present study, we propose to analyse effect of SCD extinction on cell survival and the implication of AMPK pathway in different human cancer cell lines.
Material and Methods: Human adenocarcinoma colic SW480 and osteosarcoma U2OS cells were transfected with siRNA directed against SCD1. Validation of SCD1 extinction was carried out 72h after transfection by HPLC analysis of [^{14}C] stearic acid conversion into [^{14}C] oleic acid in intact cancer cells (desaturation level). We measured protein expression by western-blot, cell viability by Cyquant® and caspase 3 activity by cytometry.
Results: Extinction of SCD1 expression in U2OS and SW480 led to a drastically reduced SCD1 activity with 3% and 4.5% of desaturation level respectively compared to about 35% in the control cells. For U2OS, abolition of SCD1 expression induced a viability decrease (almost 50%) and about 30% of SCD1-depleted cells are positive for active caspase3. We also observed PARP cleavage in depleted SCD1 cells confirming activation of apoptotic pathway. Cell death could not be prevented by addition of 100 μM of oleic acid – a product of SCD1 activity – in depleted SCD1 cells. Then, de novo MUFA synthesis appeared necessary to cancer cell survival. We demonstrated that here the AMPK pathway is activated in depleted SCD1 cells.
Conclusion: MUFA biosynthesis pathway appears as a promising target for cancer therapy since extinction of SCD1, the rate limiting enzyme of MUFA synthesis, leads to cell death of cancer cells.

445 Study of the molecular mechanism of LIF induction by TGF-beta

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Glioblastoma (GBM) is the most common tumour of the adult brain, and it is one of the deadliest tumours, with a median survival of 15 months, despite of the therapies. Because of that, it is of outmost importance to underlie the molecular mechanisms that drive the glioma progression, aggressivity and recurrence, in order to find new treatments.
Recently, our group has demonstrated the importance of the cytokine TGF-beta in glioma progression, showing that those patients with an increased TGF-beta pathway activity have worse prognosis. We are focused in the study of the molecular mechanisms that drive this oncogenic effect of TGF-beta. We want to underlie which are the mediators of this oncogenic effect, and one important mediator is the cytokine LIF (Leukemia Inhibitory Factor). We have demonstrated that the induction of LIF by TGF-beta is crucial for the Glioma Initiating Cells (GICs) self-renewal, enhancing the tumour formation and recurrence. We are especially interested in studying the molecular mechanisms of LIF induction by TGF-beta, as not all the tumours induce LIF in response to TGF-beta.
We studied the LIF promoter region searching for putative transcription factor binding sites, to find possible partners that cooperate with TGF-beta pathway in the LIF induction. We found a putative Runx1 binding site, and we are studying the role of this transcription factor in LIF induction by TGF-beta. We are also interested in its role as an oncogene in GBM.
We are postulating that the Runx1 transcription factor is necessary for LIF induction in response to TGF-beta, so its expression is crucial for tumoural cells in order to increase its self-renewal capacity. We are further studying the role of Runx1 in tumourogenesis and its importance in glioma. Our hope is that, the knowledge about the molecular mechanisms that are involved in the gliomagenesis, will lead us to develop further therapies against this outmost uncurable disease.

446 ETV5 promote epithelial to mesenchymal transition during endometrial carcinoma invasion and is modulated by LPP

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Background: This study aims to characterize the mechanisms of invasion of the endometrial cancer (EC) by focusing on the role of ETV5 and LPP.
Methodology: Differentially expressed proteins were identified by DIGE analysis. In vitro studies were carried out using Hec1a cell line, and its stable clones of GFP-ETV5 upregulation (HGE) and LPP knockdown (sLHGE). We performed immunofluorescence, western blotting and functional assays as videomicroscopy, luciferase and adhesion assays. Chromatin immunoprecipitation was used to identify targets of ETV5. cDNA Microarrays broad our understanding on ETV5 and LPP effects.
Results: Hec1a cell line grows in compact colonies, with well-defined cell-cell contacts. On HGE, cells become disperse, showing a typical mesenchymal phenotype. We report how ETV5 overexpression is able to disrupt cell-cell contacts by decreasing protein and/or mRNA levels of structural proteins, as E-Cadherin at adherens junctions, ZO3 and Claudins at tight junctions and Plakophilin at desmosomes. Furthermore, other proteins localized at contacts like the immunoglobulins and integrins are modified. ETV5 also promote the expression of mesenchymal markers like N-Cadherin or Fibronectin. All these effects are associated with a 2-fold increase rate of migration in HGE. On a first approach to Epithelial-Mesenchymal transition (EMT) we describe how ETV5 is capable to bind ZEB1 promoter, known repressor of E-Cadherin. In addition, we also observe that HGE are more proliferative and more adherent to different matrices than Hec1a. LPP was identified as a protein up-regulated in the invasive stage of EC. We describe how LPP is localized mainly at cell-cell adhesions in Hec1a, and surprisingly, it is relocalized mainly to focal adhesions in HGE. We associate LPP relocalization pattern with ETV5 capability to promote invasion, since transcription based luciferase studies and migration assays on sLHGE revert the increased luciferase expression and increased migratory ability of HGE.
Conclusions: ETV5 overexpression can promote EMT by disrupting cell-cell contacts and increase mesenchymal markers, and also, promote adhesion, increase migration and induce proliferation. Hence, ETV5 would confer to the tumour the invasive capabilities needed to disseminate. In addition, LPP might be a novel coregulatory partner for ETV5 and its relation links LPP to a communication pathway between cell-cell contacts and the nucleus, and implicates LPP in ETV5-associated functions.

447 A story of complexity and discrepancy: CD133 expression and tumourigenicity of colon cancer cell line subpopulations

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Background and Aim: Increasing evidence supports the hypothesis of tumour-initiating/cancer stem cells (TIC/CSC) in solid tumours to relate to poor prognosis and recurrence of disease. The study of cancer subpopulations with exclusive TIC potential is challenging because of the imperfect tools to isolate TIC/CSC, the controversial discussion on the culture methods for their expansion and the divergence in *in vivo* tumourigenicity in diverse animal models. Another subject of fierce debate is the potential of established cell lines to reflect CSC/TIC behavior. CD133 is a biomarker described to identify and/or enrich CSC/TIC from both primary colorectal cancers (CRC) and the established cell line HT29. This could not be verified in other CRC cell lines. Because of the discrepancy, we isolated CD133^{+} and CD133$^{-/low}$ populations from our HT29 cell pool and analyzed *in vitro* survival under defined (treatment) conditions as well as *in vivo* tumourigenicity.
Materials and Methods: CD133^{+}/CD133$^{-/low}$ HT29 and HCT-116 populations were isolated via FACS. 2-D colony formation assays were performed to evaluate cell survival under various milieu conditions (lactate, acidosis) and response to treatment (irradiation, 5-FU, oxaliplatin). 3-D spheroid formation and growth was monitored and *in vivo* tumourigenicity was evaluated in an NMRI (nu/nu) mouse model. CD133 expression was verified by flow cytometry and/or western blotting.