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

M. Minville[1], A.S. Pierre[1], J. Gresti[1], C. Fèvre[1], J. Bellenger[1], S. Bellenger[1], L. Pichon[1], M. Narce[1], M. Rialland[1]. [1]*UMR866 – INSERM, Cancer Lipides Nutrition, Dijon, France*

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

A. Gonzalez Junca[1], J. Seoane[1]. [1]*Vall d'Hebron Institute of Oncology (VHIO), Gene Expression and Cancer, Barcelona, Spain*

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

E. Colas[1], A. Ruiz[1], M. Monge[1], M. Gonzalez[1], M. Llaurado[1], J. Castellvi[2], J. Reventos[1], E. Friederich[3], D. Vignjevic[4], M. Abal[1]. [1]*Hospital Valle de Hebron, Biomedical Research Unit, Barcelona, Spain,* [2]*Hospital Valle de Hebron, Department of Pathology, Barcelona, Spain,* [3]*University of Luxembourg, Life Science Research Unit, Luxembourg, Luxembourg,* [4]*Institut Curie, UMR 144, Paris, France*

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

Y. Garbe[1], C. Dittfeld[1], S. Peickert[1], J. Waurig[1], A. Dietrich[1], M. Grade[2], T. Ried[3], L.A. Kunz-Schughart[1]. [1]*OncoRay – Center for Radiation Research in Oncology TU Dresden, Tumour Pathophysiology, Dresden, Germany,* [2]*University Medicine Goettingen, Department of General and Visceral Surgery, Goettingen, Germany,* [3]*National Cancer Institute National Institutes of Health, Section of Cancer Genomics, Bethesda, USA*

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.

Results and Conclusions: In contrast to HCT-116, $CD133^{-/low}$ HT29 cells showed a lower clonogenic survival and reduced spheroid formation capacity than their $CD133^{+}$ counterparts. HT29 cell survival decreased in a lactate-enriched milieu, an effect that was more pronounced in the $CD133^{-/low}$ population indicating that $CD133^{+}$ cells may better survive in a pathophysiological environment. All differences were significant but not as pronounced as expected. Also, no difference in response to treatment was observed for the different populations, and tumour formation capacity was 100% for as low as 500 cells injected s.c. per animal. We therefore analyzed CD133 expression after sorting and found a clear, yet unexpected rapid increase of the $CD133^{+}$ fraction in the $CD133^{-/low}$ sorted HT29 population in 2-D and 3-D culture under serum-supplemented conditions. The mechanisms of CD133 expression control have to be elucidated to verify if CD133 in CRC cell lines and tissue may be an epiphenomenon of environmental conditions.
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448 Targeting the p53 tumour suppressor activity in Glioblastomas using small molecule MDM2-inhibitor

P. Monfared[1], D. Rudan[1], L. Franken[1], G. Schneider[1], T. Viel[1], M. Hadamitzky[1], Y. Waerzeggers[1], A.H. Jacobs[1]. [1]*Max Planck Institute for Neurological Research, Neurology, Cologne, Germany*

Introduction: Targeted therapies that inhibit the MDM2-p53 interaction and the downstream Rb-E2F signalling pathway have shown promising anticancer activity, but their efficacies in human glioma have not been investigated. Recently, small-molecule antagonists of MDM2, the MDM2-inhibitiors, have been developed to inhibit the MDM2-p53 interaction and to activate p53 signalling serving possible anti-cancer activity.
Aim: To investigate the therapeutic potential of disrupting the MDM2-p53 interaction in human glioma cells with various p53 status. We particularly followed whether MDM2-inhibition would sensitize gliomas to additional chemotherapy.
Methods: We investigated the activity of MDM2-inhibitor alone and in combination with chemotherapy on cell cycle regulating proteins by Western blot and *in vivo* by employing imaging sensing vectors.
Results: MDM2-inhibior alone and in combination with BCNU results in a dose- and time-dependent reduction in cell viability and proliferation. Western blot studies showed that MDM2-inhibition modifies expression of several genes and results in cell cycle arrest and induction of apoptosis. Moreover, we found consistent and robust accumulation of p53 protein and downregulation of E2F-1 protein triggered by MDM2-inhibition alone and in combination with BCNU in all glioma cells as well as primary glioma samples. The MDM2-inhibitor and BCNU mediated alteration of p53 and E2F1 activities could be quantified in vivo by bioluminescence imaging and correlated to our results in culture.
Conclusions: Our results demonstrate that MDM2 inhibition elicits a dose- and time-dependent antiproliferative effect of glioma growth and potentiates the effects of BCNU via p53-dependent and p53-independent mechanisms and multiple genes seem to be involved in this process. MDM2 inhibitors with broad spectrum of antitumour activities in human cancers regardless of p53 status, may provide novel approaches to the therapy of malignant brain tumours.
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449 Inhibition of vascular-like network formation of highly aggressive melanoma

O. Itzhaki[1], E. Greenberg[1,2], M.J. Besser[1], Y. Nemlich[1], R. Ortenberg[1,2], J. Schachter[1], A. Treves[1], G. Markel[1,2,3]. [1]*Ella Institute of Melanoma, Sheba Cancer Research Center, Tel Aviv, Israel,* [2]*Departments of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Israel,* [3]*Talpiot Medical Leadership Program, Sheba Medical Center, Tel Aviv University, Israel*

Melanoma cells display substantial plasticity, demonstrated by directly forming tube-like structures composed of tumour cells but not of endothelial cells, which conduct blood cells and fluid. This phenomenon was termed Vasculogenic Mimicry (VM). Recently, it was shown that the presence of VM in melanoma masses predicts poor prognosis. Noteworthy, several anti-angiogenic lines of therapy seem ineffective against melanoma. It could be speculated that this alternative vascularization pathway might be of importance for advancement of melanoma.
We examined the ability of two agents to abrogate VM: IFNalpha, an immunomodulator with an antiangiogenic effect, and nicotinamide, the amide form of vitamin B3 (niacin).
The in vitro effects of the agents were examined using the highly aggressive melanoma cells (C8161). VM was tested as formation of tubular networks when grown in three-dimensional (3D) culture. In addition, cell proliferation (measured with XTT), cell cycle analysis (DNA content) and invasion capacity through matrigel were tested concomitantly.
IFNalpha affected *in vitro* VM formation in a dose-depended manner (at concentrations of 5×10^4 and 5×10^5 IU). Further, IFNalpha significantly inhibited the proliferation of C8161 cells. Cell cycle analysis reveled a significantly increased proportion of apoptotic cells. Moreover, the invasion ability was decreased in the treated cells. Nicotinamide (at concentrations of 1 and 5 mM) significantly inhibited the proliferation of the melanoma cells, but had no effect on their invasion capacity. According to cell cycle analysis, nicotinamide treated cells showed no significant changes in their respective apoptotic indices. Nicotinamide inhibited VM formation, but the effect was inconsistent. All effects were compared to control treatments with carrier only.
Due to the fact that both IFNalpha and nicotinamide hold a wide range of biological activity, the dose for optimal results may differ greatly as different effects are mediated by different concentrations. Nevertheless, both demonstrated anti-melanoma properties, including an effect on VM formation. Targeting VM could be of great importance, especially in combination with anti-angiogenic strategies. This combination is expected to be synergistic and yield substantial anti neoplastic effect.

450 Leptin and estrogen receptor expression in breast cancer patients with different clinical characteristics

A. Lushnikova[1], E. Peredereeva[1], L. Lyubchenko[2], A. Parokonnaya[3], E. Polevaya[4]. [1]*N.N. Blokhin Russian Cancer Research Center, Oncovirus Immunology Laboratory, Moscow, Russian Federation,* [2]*N.N. Blokhin Russian Cancer Research Center, Clinical Genetics Laboratory, Moscow, Russian Federation,* [3]*N.N. Blokhin Russian Cancer Research Center, Radiosurgery Department, Moscow, Russian Federation,* [4]*N.N. Blokhin Russian Cancer Research Center, Mammary Tumours Department, Moscow, Russian Federation*

Introduction: Leptin is a multifunctional hormone produced by adipocytes. It plays important role in angiogenesis. Induction of cell proliferation, survival and anchorage-independent growth.
These leptin activities are mediated through leptin receptor (ObR) that binds leptin molecule and stimulates Jak/STAT 3, ERK 1/2, cyclin D1 expression and other signal pathways. A recent data show that targeting leptin signaling may reduce mammary carcinogenesis and breast cancer (BC) progression. However, the link between obesity and leptin expression in serum/breast tumour as well as its role in modulation of estrogen receptors (EsR) and HER2/neu expression is not clear.
Material and Methods: We studied leptin, ObR, EsR, HER2/neu expression in patients with sporadic, familial and pregnancy-associated BC by RT PCR using BC fresh tissue and primers for genes encoding leptin, ObR, EsR-α, β. Leptin level in the patient sera was estimated also by ELISA (Leptin Sandwich DRG, DRG Diagnostics, Germany) followed by comassie staining. The data on routine immunohistochemical staining of BC paraffin embeded section for HER2/neu, EsR and PrR were also obtained. In control group were patients with benign fibroadenoma (BFa) and healthy women of comparable age.
Results: RT PCR results and immunohistochemistry method are mainly concordant: only 5% (5/29) of data were different. In triplonegative tumours (n = 40) leptin overexpression was significantly higher than in other tumour types. Blood sera leptin level was correlates positively with ObR expression. Leptin expression in tumour tissue also correlates with HER2/neu over expression in all BC groups except triplonegative ones: Esr (−), PrR(−), HER2/neu(−). Serum leptin levels in BFa patients was higher (100–300 ng/ml) than in healthy women of comparable age and body weight. Moreover, leptin serum level was positively correlates with ObR expression in tumours on I-III BC stages and with high body weight index (obesity).
Conclusions: The data obtained indicate that leptin/ObR may involved in BC progression. It indicates that ObR suppression is the possible way for target BC therapy, especially of triplonegative tumours which do not express HER2/neu, so hormone therapy is not effective for these neoplasia.

451 Progesterone regulation of breast cancer cell coagulative and invasive potential is dependent on the distinct membrane localization of tissue factor

G. Owen[1], M.L. Bravo[1], M. Quezada[1], S. Henriquez[1]. [1]*Pontificia Universidad Catolica de Chile, Physiology, Santiago, Chile*

Background: The oncogene Tissue Factor (TF) is over-expressed in breast cancers and is correlated to metastasis and thus poor prognosis. The usage of exogenous progestins is associated with increased breast cancer incidence. We previously reported that TF is transiently regulated by progesterone at the level of transcription and that the blocking of TF activity by antibodies eliminates the progesterone-mediated coagulative and invasive potential of the breast cancer cell lines ZR-75 and T47D.
Material and Methods: Coagulation was measured in whole cells by the generation of FXa in the presence of FX and FVIIa. Invasion was measured