was associated with release of danger signals including HMGB1 and Heat shock proteins. *In vivo*, Dox treatment resulted in tumour regression which was reduced in immune deficient compared to immune competent mice. Pentamers were used to measure the specific T cell response and infiltration of immune effector cells were analysed by IHC.
Conclusions: This system allows us to explore the relationship between the amount and type of cell death and the ability to prime tumour-(ova)-specific T-cell responses *in vivo;* provide important clues as to what regulates immunogenicity of cell death *in vivo*; and eventually guide therapeutic approaches which aim to induce immune responses to dying tumour cells.

398 Glycan gene expression signatures distinguish normal and malignant breast tissue; possible role in diagnosis and progression

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Background: Glycosylation is the stepwise procedure of covalent attachment of oligosaccharide chains to proteins or lipids, and alterations in this process have been associated with malignant transformation. Studies focusing on the expression of the whole glycome have now become possible and prompted us to perform a comprehensive analysis of breast carcinomas focusing on glycosylation related genes.
Material and Methods: Various data resources were used to select a set of 419 functionally relevant genes. Two expression data sets were analyzed. The first consisted of samples from 64 stage I-IV breast cancer patients and normal breast tissue from 79 healthy women. Additionally, expression data from tumour and adjacent normal tissue of 26 breast cancer patients was analyzed.
Results: The glycome mRNA expression pattern was significantly different in tumour tissue compared to normal breast tissue, demonstrating the involvement of glycosylation in malignant transformation at several levels. The N-glycan pathway seems to be affected at different stages involving both the early precursor synthesis as well as certain later modifications including β1,6 branching and addition of α1,6 fucose to the core. Such reconfiguration may have a modulating effect on signaling of integrins, cadherins, epidermal growth factor and transforming growth factor-β leading to changes in growth pattern and possibly playing a role in the epithelial-mesenchymal transition. Furthermore, expression of glycosyltransferases involved in the synthesis of glycosphingolipids implied a profound change in structural appearance of gangliosides, including differences in sialylation. These changes may result in alteration of intercellular adhesion and signaling. Transcription levels of O-glycan related genes point to an altered glycosylation of mucins which in turn may influence adhesion and immunogenic properties of carcinoma cells. The same might be achieved through alterations in Lewis antigen structures presented on the cell surface as suggested by altered mRNA levels of a variety of fucosyl, sialyl- and galactosyltransferases, indicating higher levels of type 2 structures. Altered expression of genes coding for transferases associated with synthesis and sulfation of several types of glycosaminoglycans may imply an impact on the local environment immediate to the cell surface, both in terms of adherence and change in the reservoir of chemokines and other signaling molecules.
Conclusion: In this study we have performed a comprehensive analysis of all known glycan-related genes using expression data from breast carcinomas and normal breast tissue samples. The results clearly demonstrate a unique glycan gene expression signature of malignant carcinomas of the breast significantly different from that of healthy breast tissue. Several of the alterations in the glycosylation pathways revealed by this signature are novel and warrant further investigation.

399 Endoplasmic reticulum stress mediates cell death in human hepatocellular cancer cells: an alternative apoptotic pathway induced by the pan-deacetylase inhibitor panobinostat

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Background: Panobinostat (LBH589), a pan-deacetylase inhibitor, represents a novel therapeutic option for human cancer diseases. We have previously shown that panobinostat has a potent apoptotic activity *in vitro* and causes a significant growth delay of hepatocellular carcinoma (HCC) tumour xenografts in nude mice models. We have demonstrated that treatment with panobinostat is able to induce cell death in HepG2 (p53wt) and in Hep3B (p53null) cell lines that, interestingly, is not dependent on canonical apoptotic pathways. Here we analyse the involvement of Endoplasmic Reticulum (ER) in cell death induced by panobinostat treatment.
Material and Methods: Human HCC cell lines HepG2 and Hep3B were cultured under standard conditions and treated for 6−72 hours with 0.1 μM panobinostat. Sub-G_1 events were quantified by flow cytometry after propidium iodide staining and verified by immunofluorescence of cytokeratin-18 cleavage. ER-stress factors were evaluated by quantitative RT-PCR and western blotting. Caspase-12 and caspase-4 activities have been determined by a Fluorometric Assay kit (Biovision), caspase-3/7 and -8 activities have been evaluated by Caspase Glo assay kit (Promega).
Results: Treatment of both HCC cell lines induced cell death as was shown by an increase in sub-G_1-events. The ER response involvement was clarified by IRE1-alpha, BIP and ATF-4 transcript evaluations that increased after 6 hours of treatment *in vitro* and after 1 day in xenografts specimens. Neither HCC cell line showed an expression of IRE1-beta, the IRE1-alpha homologous gene. Moreover, panobinostat caused an increase of expression for CHOP/GADD153 transcript and a stable expression of its protein level; otherwise a decrease in the level of Xbp transcript in HepG2 cells was shown. We also demonstrated the up-regulation of eIF2-alpha phosphorylated form after treatment with panobinostat in Hep3B cells. A transient increase of the phosphorylated status of JNK, ERK and p38MAPK was clearly detected. Finally, activation of caspase-12 and caspase-4 was detected and their inhibitions lead to a downregulation of caspases-3/7 and -8 activities.
Conclusion: The novel pan-DACi panobinostat induces cell death in HCC cell lines. ER-stress plays a key role to drive cells to die through the induction of three main actors of alternative death pathway: CHOP, JNK and caspase-12/-4 leading to activation of executioner caspases.

400 Met as a potential therapeutic target in basal-like breast cancer

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Met overexpression has been associated to a highly invasive and poorly differentiated subtype of breast cancer, known as basal-like breast cancer. These tumours show an aggressive phenotype and do not express hormone receptors or Erb2, which makes them insensitive to therapies currently in use for mammary tumours. Based on gene expression profiles, it has been proposed that basal-like tumours derive from mammary stem/progenitor cells. The deregulation of pathways specific to mammary undifferentiated cells may contribute to the generation of these tumours. We investigated how the MET affects function of normal mammary cells, and whether its hyperactivation favors to the formation of neoplastic lesions.
We explored the functional role of Met expression in mammary gland development by fat pad transplantation experiments. Constitutive activation of Met in the transplanted cells enhanced their proliferation ability with the formation of a hyper-branched ductal tree and dilations of the TEBs. In limiting dilutions transplants, Met activation led to a significant increase in the frequency of mammary repopulating units compared to wild-type cells.
Consistently, in vitro cultures showed that hyperactivation of Met in primary mammary cells induced the generation of colonies higher in number and larger in size than those arising from wild-type cells; moreover, Met pharmacological inhibition reduced the growth potential of mammary cells on irradiated fibroblasts, underscoring the role of Met in sustaining the clonogenic ability of mammary cells. Gene expression analysis and flow cytometry-based cell sorting revealed that Met is differentially expressed in the various mammary epithelial subpopulations: it is highly expressed in luminal progenitors ($CD24^{high}$ ER^{-}), whereas it is barely detectable in the differentiated cells of the basal $CD24^{low}$ compartment – which also includes stem cells – and in the terminally differentiated luminal cells $CD24^{high}$ ER^{+}. Interestingly, the $CD24^{high}$ ER^{-} progenitor population has been recently described as the candidate target population for basal tumour development in BRCA1 mutation carriers. Expression analysis in tumours derived from a mouse model of basal-like cancer (BRCA1/p53 ko) revealed that Met is overexpressed in a subpopulation of CD24+ ER− cells. This is in line with the observation that Met overexpression in basal-like breast tumours might play a causative role in the onset and maintenance of the transformed phenotype.

401 A new Golgi-based signalling cascade involved in tumoural cell invasion

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Background: Metastasis is the most frequent cause of death in cancer patients but the molecular mechanisms that regulate metastatisation remain to be clearly defined. We have demonstrated that KDEL receptor (KDELR) engagment by incoming traffic at the Golgi complex triggers activation of the oncogenic Src family kinases (SFKs) on the Golgi itself. The aim of this study is to determine the role of this new signalling pathway in tumoural cell invasion.

Materials and Methods: To define the role of the KDELR-Golgi-SFK signalling cascade in cell invasion I assessed the ability of A375MM melanoma cells to degrade the extracellular matrix (ECM). Cells were transfected with different constructs to activate/inhibit KDELR and then plated on a fluorescent gelatin matrix in the presence of BB94, a broad spectrum metalloprotease inhibitor. After 16 hours, BB94 was washed out and cells were fixed after 3 hours and processed for immunofluorescence. The degradation areas, visible as dark "holes" in the fluorescent gelatin matrix, were quantified by the LSM510−3.2 software (Zeiss).
Results: The chronic activation of the KDELR by either over-expression of the KDELR itself, which induces its autoactivation, or transfection of artificial secretory proteins that act as KDELR agonists increases ECM degradation. In contrast, the over-expression of the KDELR mutant KDELR-D-193-N leads to a decrease in ECM degradation. The increase in ECM degradation correlates with an increase in SFK activation. Moreover, upon KDELR activation there is an increase in the phosphorylation levels of two SFK substrates, the ARF GTPase activating protein ASAP1 and the focal adhesion kinase FAK. These data indicate that KDELR-Golgi-SFK signalling affects ECM degradation through the involvement of ASAP1 and FAK.
Conclusions: We have investigated whether KDELR–Golgi–SFK signalling could regulate tumour cell invasion. Our data indicate that the chronic activation of this cascade promote ECM degradation. We believe that the Golgi complex has a crucial role in cell invasion, not only by providing hydrolytic enzymes for ECM degradation but also through KDELR-initiated-signalling. Our investigation is important because it highlights a new signalling cascade involved in the regulation of cell invasion thus expanding our knowledge of the metastatisation process and possibly providing new druggable targets to exploit for anti-metastasis therapy.

402 The endogenous EPO/EPOR system contributes to glioma cell proliferation both in vitro and in vivo

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Background: The biology of erythropoietin (EPO) has been recently re-evaluated following the discovery of its receptor (EPOR) on numerous cancer cells [1]. Concerning brain tumours, although the expression of the EPOR has been described on glioma cells [2], data from the literature remain descriptive and controversial [3] and to date no clear demonstration of a potential effect of EPO on controlling tumour growth has been described. Accordingly, the aim of this study was to evaluate and compare both in vitro and in vivo, the effect of two complementary strategies developed to block the biological effect of EPO/EPOR on glioma cell growth.
Material and Methods: Human U87 and U251 glioma cells were genetically modified by RNAi to stably invalidate EPO or EPOR expression. Full-length EPOR (9L-EPORF) or a truncated variant used as a negative dominant for EPOR (9L-EPORT) were stably overexpressed by rat 9L glioma cells. Effects of genetically modified cells were studied both in vitro on cell proliferation by automatic cell counting and in vivo by a longitudinal MRI follow-up of tumour growth and a survival study after intrastriatal implantation of these cells in Fischer rats (for 9L cells) or nude mice (for U87 and U251 cells).
Results: On one hand, the invalidation of either EPO expression (with shRNA EPO) or EPO availability (with soluble EPOR) on distinct human glioma cell lines led to a sustained decrease in cell proliferation from day 1 to day 4 (at day 4, U87-shEPO: 27±5% versus U87 control, $n = 3$, $p < 0.05$). On another hand, the invalidation of EPOR expression or EPOR signaling also induced a reduction of glioma cells number in vitro. Accordingly, in vivo, we observed on day 34 a decrease in tumour volume for the mice bearing U87-shEPOR ($59 \pm 16\ mm^3$), as compared to U87-Control mice ($107 \pm 30\ mm^3$; $n = 4$ for each group, $p = 0.05$). In addition, we also measured by MRI at day 13, a significant reduction of the tumour volume for animals bearing 9L-EPORT cells ($5.4 \pm 1.5\ mm^3$) in comparison to control rats (9L cells: $77.7 \pm 34.8\ mm^3$, $n = 4$ both groups, $p < 0.05$). An increase in the animal survival median was also observed for 9L-EPORT (28 days) in comparison to 9L rats (21 days, $n = 6$ both groups, $p < 0.05$).
Conclusions: Collectively, by the use of two strategies of EPO/EPOR inhibition on glioma cells, our results support that the autocrine EPO/EPOR loop on tumour cells might be a critical mediator of cell proliferation which might influence tumour outcomes of patients with brain tumours.
Supported by a grant from INCa (Institut National du Cancer) and the Institut Lilly. Authors thank Lundbeck A/S (Copenhagen, Denmark) for the gift of rhEPO.

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403 Mitochondrial superoxide dismutase overexpression changes neuroendocrine, proliferation and apoptosis resistance features in prostate cancer cells

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Prostate cancer (PC) is a tumour greatly associated with aging and oxidative stress one of the most accepted theories to explain aging. Therefore antioxidant enzymes such as MnSOD/SOD2 have been studied in relation to androgen-independent transition and resistance to radio/chemotherapy treatments. The main role of MnSOD is superoxide radical scavenging and thus this mitochondrial enzyme is induced, among other stimuli, as a radiation-adapted signal. Neuroendocrine (NE) cells are postmitotic cells and the less frequent cell type in the prostate gland that show androgen independence and resistance to treatment. Interestingly, NE differentiation is a common process along with PCa progression.
In order to study the role of MnSOD in NE cells, we stably overexpresed androgen dependent LNCaP. In these clones we first confirmed the presence of NE markers and apoptotic-related proteins by western blotting. Then we tested (1) proliferation rate; (2) the ability of cells to grow in androgen deprived medium both by cell counting; (3) apoptosis resistance to chemotherapeutic treatment by MTT viability assay; and (4) the potential capacity of MnSOD-expressing cells-conditioned media of inducing cell growth in androgen independent PC-3 cells by DNA staining with Hoechst.
MnSOD overexpressing LNCaP clones, namely MnSOD-S4 and S12, show typical NE morphology when compared to mock clones (pcDNA-P2), which was also confirmed by levels of Synaptophisin, a widely used NE marker. Surprisingly, a higher proliferation rate was observed in MnSOD-S4 and S12 clones, indicating that MnSOD overexpression keeps proliferation capacity. In androgen-stripped media, MnSOD-S12 clone displays a significantly higher proliferation rate than pcDNA-P2 while protein levels of androgen receptor are decreased in both, MnSOD-S4 and S12 clones. Regarding apoptosis resistance, MnSOD-S12 clone showed higher resistance etoposide-induced cell death Finally, PC-3 cells maintained with conditioned medium obtained from MnSOD-S4 showed higher levels of proliferation compared with pcDNA-P2.
The increase of MnSOD expression is enough to induce most of the reported morphological and biochemical characteristics of androgen-independent NE-like cells in PC. This would indicate that redox balance mediated by MnSOD could be a key step in androgen dependent-independent transition in PC.

404 A novel role for JAM-A as a crucial regulator of breast cancer cell motility through downstream effects on Rap1GTPase and Beta1-integrin

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Introduction: The cell-cell adhesion protein junction adhesion molecule-A (JAM-A) influences epithelial cell morphology and migration. We have previously demonstrated that high JAM-A levels in breast cancer tumour cells are significantly associated with poor prognosis in breast cancer patients (McSherry, 2009). In addition, we have recently shown that functional inhibition or knockdown of JAM-A decreases MCF-7 breast cancer cell migration, likely due to reduced levels of β1-integrin. The aim of our ongoing studies is to define the pro-migratory signalling cascades at play in breast cancer cells from JAM-A (at the cell-cell interface) to β1-integrin (at the cell-matrix interface).
Materials and Methods: MCF7 cells following siRNA-mediated JAM-A gene knockdown (JAM-A KD cells) were investigated via western blot and immunoprecipitation analysis to determine protein expression of putative signalling pathway proteins (downstream of JAM-A). In addition, JAM-A KD cells were assayed for alterations in cancer cell migration and cell adhesion. As Rap1 is a known activator of integrins, cell migration of MCF7 cells was determined following treatment with either a Rap1GTPase pharmacological inhibitor or a b1-integrin inhibitory antibody. Analysis of active Rap1 was performed in MCF7 cells following either JAM-A gene knockdown or JAM-A protein antagonism. Affects of JAM-A antagonism on PDZGEF2 (a crucial Rap1 activator) was assessed by immunofluorescence and western blot analysis.
Results: We have demonstrated that following JAM-A gene knockdown the protein expression of Rap1 and the Rap1 regulator AF-6 is decreased, and direct association of JAM-A and AF-6 is altered. In addition, the activity of Rap1 is reduced upon either JAM-A knockdown or inhibition. Furthermore, we demonstrated that breast cancer cell migration is decreased upon inhibition of both Rap1GTPase and b1-integrin. Finally, we demonstrate that PDZGEF2 localisation is altered following JAM-A inhibition.
Conclusion: We suggest that JAM-A over-expression can initiate a signalling cascade involving AF-6, PDZGEF2, Rap1GTPase and b1-integrin, to promote