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

I.O. Potapenko[1], V.D. Haakensen[1], T. Lüders[2], A. Helland[1], I. Bukholm[2], T. Sørlie[1], V.N. Kristensen[1], O.C. Lingjærde[3], A.L. Børresen-Dale[1]. *[1]Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Department of Genetics, Oslo, Norway, [2]Institute for Clinical Epidemiology and Molecular Biology (Epi-Gen), Faculty Division Akershus University Hospital, Faculty of Medicine, Oslo, Norway, [3]Institute for Informatics, Faculty of Natural Sciences and Mathematics, University of Oslo, Oslo, Norway*

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

R. Montalbano[1], P. Di Fazio[1], T. Wissniowski[2], S. Gahr[2], K. Quint[1], M. Ocker[1]. *[1]Universitätklinikum Marburg, Institute for Surgical Research, Marburg, Germany, [2]Universitätklinikum Erlangen, Department of Medicine, Erlangen, Germany*

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

S. Gastaldi[1], A. Bertotti[1], F. Galimi[1], F. Sassi[1], D. Torti[1], M. Smalley[2], L. Trusolino[1]. *[1]Irrc, Molecular Oncology, Torino, Italy, [2]Breakthrough Breast Cancer Research, Institute of Cancer Research, London, United Kingdom*

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

C. Ruggiero[1], M. Grossi[1], M. Capitani[1], R. Buccione[1], A. Luini[1], A. Luini[1], M. Sallese[1]. *[1]Consorzio Mario Negri Sud, Department of Cell Biology and Oncology, S. Maria Imbaro, Italy*

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.