different DNA repair pathways and also of links between DNA repair and cell cycle. Mathematical modelling of the network will bring a better understanding of dynamic regulatory circuits between these two mechanisms. The network will be used for discovering key players in breast cancer progression and will help identifying potential new therapeutic targets.

827 Deciphering the crosstalk between Hh/GLI and other signaling pathways implicated in cancer development and progression

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Sustained Hh/GLI activity is one of the main driving forces responsible for basal cell carcinoma (BCC). BCC is the most common form of skin cancers occurring approximately in about 30% of Caucasians during their lifetime. Besides, an increasing number of scientific reports indicate that a malfunction of the Hedgehog pathway also contributes to other malignant diseases such as lung, prostate, gastric and pancreatic cancer. Therefore, it is of considerable interest to get a precise understanding of the exact molecular mechanisms controling the different aspects of Hh/GLI signaling.
The downstream effectors within Hh pathway are the GLI transcription factors which directly control the expression of specific target genes. Several recent publications point to the fact that the activity of GLI proteins is influenced by a multitude of other signals, for example from ERK and AKT signaling. However, there is only insufficient knowledge how the finely tuned interplay between the different pathways modulates the complex pattern of GLI target gene expression.
Protein microarrays are a large-scale tool which provide quantitative information on dynamic processes and were therefore chosen as experimental platform to shed light on the crosstalk between Hh signaling with other cancer-relevant signaling pathways. Data obtained on the dynamics of the (phospho-) proteome together with data generated through transcript profiling will be integrated into a computational model of Hh signaling. Based on this model predictions on how a cell reacts under specific conditions will become possible. Finally, the interplay between biological experiments and computational modeling has great potential to improve our understanding of cancer induced by aberrant Hh/GLI signaling and promises to aid future drug discovery strategies. The project (MOGLI) is funded bi-nationally by BMBF and Gen-AU as part of the Medical Systems Biology Call.

828 Ewing sarcoma network model through EWS-FLI1 signaling

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Background: Ewing's sarcoma is characterized by frequent chromosomal translocation t(11;22)(q24;q12) which leads to appearance of a chimeric oncogene EWS-FLI1. This molecule acts as an aberrant transcription factor and causes a cascade of deregulations triggering tumourigenesis. We report on studying systemic effect of EWS-FLI1 on apoptosis and proliferation phenotypes.
Material and Methods: *Experimental models*: inducible Ewing tumour cell lines were constructed, where EWS-FLI1 expression can be controlled. Transcriptome time series following inhibition and rescue of EWS-FLI1 expression were measured for these cells; in addition, for a selected group of genes, siRNA-based gene knock-downs were performed followed by measuring gene expression changes by qPCR. CHiP-Seq experiments were performed on these cells in order to study the DNA binding properties of EWS-FLI1. Microarray expressions were measured for a collection of tumour biopsies.
Theoretical methods: model based characterization of time series was set up, based on curve fitting. Original method of influence network reconstruction was applied: it integrates literature mining in Cytoscape environment (network visualization software), through BiNoM java plugin. A procedure for confronting large influence networks (>100 nodes) with data was created, based on path analysis. A reverse engineering method was set up for the analysis of gene expression shifts upon perturbations (siRNA + qPCR); it can identify new regulatory links between genes involved in EWS-FLI1 signalling.
Results: A list of genes induced or repressed in inducible cell lines was created. An influence network based on this list was constructed and recapitulated EWS-FLI1 effect on proliferation and apoptosis. This network was confronted to data (cell lines and tumour samples); network and data appear to be coherent with observed phenotypic changes induced by EWS-FLI1. This analysis allows to classify genes upon their effects on phenotypes. Analysis of qPCR experiments, including the confrontation with influence network, reveals new transcriptional regulatory relations between genes. Finally, a global gene prioritization analysis was performed on the whole corpus of high-throughput data (expression in cell lines and tumours, prediction of functional binding sites of EWS-FLI1); a list of 250 potential EWS-FLI1 targets and critical players was extracted from it, for further siRNA-based phenotypic knock-down screening.
Conclusion: Our results demonstrate that the action of the EWS-FLI1 transcription factor cannot be reduced to a single gene/pathway; therefore, EWS-FLI1 effect should be investigated in a global systemic manner, involving a number of deregulations in multiple signaling pathways through a complete gene regulation network.

829 Heat stabilization of tissue biopsies for improved cancer proteomics

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Background: From the moment of sampling, tissue biopsies are subjected to internal processes that change the proteome composition and important information about the 'pre-sampling' state may be distorted or destroyed, leading to reduced reproducibility between samples and even faulty conclusions. In this experiment a novel stabilization system was used to treat fresh and frozen tissue samples to stop degradation and preserve the *in vivo* proteome by inactivation early in the sample preparation chain.
Material and Methods: To address the problem of *ex vivo* degradation, a rapid heat inactivation system for tissue samples has been evaluated. The system stops enzymatic post-sampling changes irreversibly in tissue by utilizing rapid and uniform thermal inactivation. This enables the detection and monitoring of important post-translational modifications, such as phosphorylation of peptides and proteins. Stabilization was assessed by nano-LC-MS, MALDI, western blotting, and spectrophotometric assays on samples from brain, muscle, and liver.
Results: Inadequate sample handling normally cause an increase in degradation fragments which has been shown in these experiments. However, after immediate sample stabilization, no protein degradation fragments were detectable. The mass spectrometrically identified peptide peaks in the stabilized samples consisted of several known neuropeptides, endogenous peptides, and novel potentially biologically active peptides. Most peaks detected in an untreated group originate from proteins such as hemoglobin, cyclophilin, NADH dehydrogenase, synuclein and other highly expressed proteins. Accordingly, the assayed functions of proteases and phosphatases showed clear inactivation after stabilization. The levels of phosphorylated forms of CREB, GSK and MAPK remained unchanged after 2 hours in room temperature after stabilization treatment as the levels of the same proteins in untreated tissue decreased in only 10 minutes.
Conclusions: Usage of the rapid heat inactivation system ensures elimination of degradation and preserves the in vivo levels of proteins, peptides and phosphorylations. Rapid heat inactivation has been shown to be of special importance when labile protein phosphorylations involved in cell signaling are studied. It is showed that stability after inactivation is maintained for several hours in room temperature compared to a drastic down regulation after only 10 minutes with normal sample handling.

830 Integration of miRNAs into the ERBB network for combinatorial targeted therapy

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Aberrant ERBB receptor signaling can induce the development of many human cancers e.g. breast, lung, brain and gastric carcinomas. Particularly, EGFR and ERBB2 belong to the most oncogenic kinases in humans and are in the center of targeted therapy, but the response rate is rather low. Recently, a novel class of post-transcriptional gene regulators was discovered and named as microRNAs (miRNAs), which are ~22 nucleotides in length and regulate the expression of genes post-transcriptionally from *C. elegans* to human. Up to now, several miRNAs (e.g. miR-7 and miR-205) have been shown to target and regulate components of the ERBB network and cancer-related cellular phenotypes. Here, we aim to identify miRNAs which regulate the ERBB network and presenting them as potential drug targets for effective combinatorial targeted therapy.
We started with constructing the ERBB network including all ERBB receptor family members, downstream signaling intermediates, negative feedback regulators, key TFs and cell cycle proteins. Using a miRNA mimic library containing 810 miRNAs, we screened the effects of each miRNA on the expression levels of the ERBB network proteins in the EGFR-overexpressing MDA-MB-231 breast cancer cell line. Since miRNAs can regulate gene expression both at mRNA and/or protein level, effects of miRNAs are

quantitatively studied at the proteomic and transcriptomic levels using Reverse Phase Protein Arrays (RPPAs) and qRT-PCR, respectively. Direct targeting of miRNAs is validated by luciferase assays as well as site-directed mutagenesis, and identified miRNAs/targets will be studied in different cancer-relevant cell-based assays (e.g. invasion and BrdU/7-AAD assays). Finally, we will test the effects of miRNAs/targets in combination with several ERBB receptor-targeting drugs e.g. gefitinib or trastuzumab in different cell line models (drug sensitive vs. resistant). Hence, combining bioinformatics, functional genomics, proteomics as well as molecular and cellular biology approaches, we integrate miRNAs into the well-studied ERBB network for combinatorial targeted therapy.

831 Applying JISTIC to different stages for breast cancer

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Motivation: We wanted to compare array CGH DNA profiles of various stages breast cancer as a surrogate of the course of tumourigenesis and identify significant aberrations that may be driving the process forward at each stage.
JISTIC (http://www.c2b2.columbia.edu/danapeerlab/html/jistic.html) is a bioinformatic tool useful to analyze datasets of genome-wide copy number alteration that detects driver aberrations in cancer. JISTIC implements the standard, focal and arm-peel-off for the widely used GISTIC (Genomic Identification of Significant Targets in Cancer) algorithm developed by Beroukhim et al. (PNAS ref, 2007). We applied JISTIC to identify the regions that are likely to be driver aberrations of the carcinogenesis regions.
Material and Methods: DNA copy levels were obtained in the form of log R values from 244 K array CGH (Agilent). Total 588 profiles of pre-malignant and malignant breast tissue were gathered from five different clinical cohorts involving healthy tissues from mammographically dense breasts, breast tumours without invasive compontent (DCIS), and stage I−IV tumours.
We segmented the CGH logR by applying piecewise constant function and performed centering of the values to avoid differences by cohorts. The centered data were divided into different stages, normal, DCIS, stage I (T1), II (T2) and III (T3). JISTIC was applied to each stage. JISTIC identified significant aberration regions and peaks.
The sets of genes identified in the significant aberration regions, were subjected to GO analysis by DAVID http://david.abcc.ncifcrf.gov.
Results: The significant peaks were 20 for normal, 19 for DCIS, 103 for T1, 92 for T2 and 25 for T3, which were in focal region, in the preliminary analysis. The number of peaks increased with increasing stages from DCIS to T2 but leveled off at T3. For the genes identified in the loci of significant aberrations, we applied GO analysis. In the case of normal, phospholipid biosynthetic process for deletion was identified and no significant term for amplification. DCIS indicated C21-steroid hormone biosynthetic process for amplification, and a term for antigen processing and again lipid metabolic process for deletion. For the remaining cases, significant GO terms were 16 (T1), 11(T2), 2(T3) for amplification, and 15(T1), 6(T2), 7(T3) for deletion.
Conclusions: Applying JISTIC to different stages of breast cancer indicated some trends; specific aberrations were observed at each stage of breast cancer pointing to important biological processes. However, since the sample size for normal, DCIS and T3 was smaller than the sample size for T1 and T2, further studies will be needed to support these preliminary findings.
I would like to thank Vilde D. Haakensen, Simen Myhre and Lars O. Baumbusch for providing the CGH data.

832 Human liver regeneration and its clinical impact

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Background: Study concerning human liver regeneration (HLR) evaluates the hepatic extraction fraction (HEF) using radioisotopic methods as an indicator of HLR of patients underwent hepatectomy (HR).
Material and Methods: 95 patients with colorectal metastases (n = 69), hepatocellular carcinoma (n = 16) and other tumours (n = 10) were included. 37% underwent major hepatectomy (*MA*HR) and 63% minor hepatectomy (*mi*HR). HLR was assessed after intravenous bolus injection of ^{99m}Tc-Mebrofenin. The HEF is calculated using deconvolution analysis of first pass curve coming from scintigraphic data. We evaluated the pre-operative HEF (T0) and in the 5th day (T5) and one month after PH (T30). We considered the HEF values of 98.8±0.4% as normal.
Results: Overall patient population: the mean HEF values in our series were 97.3±9.6% for T0, 97.5±8.3% for T5 and 98.7±4.1% for T30(ns). The mean Tmax values were 15.6±8.1 min for T0, 13.9±5.7 min for T5 and 14.1±5.4 min for T30 (ns). The mean T1/2 values were 35.7±22 min for T0, 86.4±105.1 min for T5 and 39.5±19.2 min for T30 ($p < 0.0001$). Subgroup of patients treated by Major *MA*HR: For the 35 patients who underwent *MA*HR the HEF values were 97.2±5.3% (T0), 95.6±12.6% (T5) and 98.9±1.8% (T30) (ns); the mean Tmax values were 16.5±7.3 min for T0, 14.3±5.6 min for T5 and 15±4.7 min for T30 (ns); and the mean T1/2 values were 27±26.2 min for T0, 89.2±74.3 min for T5 and 42.2±8.7 min for T30. There are significant differences only for the values of T1/2 in T5 when compared with T0($p < 0.035$). For the 60 patients who underwent *mi*HR the HEF values were 97.4±11% (T0), 98.8±2.4% (T5) and 98.6±4.7% (T30) (ns); the mean Tmax values were 15.2±8.5 min for T0, 13.6±5.9 min for T5 and 13.8±5.7 min for T30 (ns); and the mean T1/2 values were 39.1±19.4 min for T0, 84.6±122.4 min for T5 and 38.6±21.6 min for T30. There are significant differences only for the values of T1/2 in T5 when compared with T0($p < 0.04$).
Conclusion: Our results strongly support the view that the HLR is early enough to normalize the HEF at day 5 after HR. We have demonstrated that HLR is early, fast, non-anatomical and functionally complete 5 days after liver resection. This fast functional liver recovery has high clinical importance because concerning adjuvant chemotherapy, possibly resulting in treatments administered much earlier after surgical resection than the three weeks dogma. Similarly, the same applies to liver resections following portal embolisation.

833 Proteomic technologies in brain tumours early diagnosis

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Background: One of the important goals of oncology is to develop biomarkers that can be identified through less invasive methods with the potential to identify cancer risk, early detection, utility in monitoring.
Several different proteomic methods have been discovered. One or a combination of methods needs to be chosen for an accurate cancer diagnosis.
Brain tumours are a heterogeneous group of neoplasms that appear to underlie individual patterns of growth, invasion, response to therapy and prognosis.
The aim of this study was to evaluate the protein profiling in brain tumours (gliomas and pituitary tumours) in order to establish a biomarker panel useful in early tumour detection.
Material and Methods: Our data were obtained using two cutting-edge proteomic profiling technologies: xMAP array – Luminex200 (cytokines panel) and SELDI-TOF-MS (protein profile). Serum samples from 3 groups: gliomas, pituitary tumours and controls were analyzed.
Results: From multiplex assay (Luminex200) strong overexpression was detected for IL-6, IL-1β, TNF-α (over 2 fold stimulation in patients vs. control). Significant up-regulation (1−2 fold) was found for VEGF and bFGF. Cytokines expression was significantly higher and strongly correlated with tumour stage, proliferation markers and clinical aggressiveness in glioblastomas and invasive pituitary adenomas. SELDI TOF proteomic profiling led to the selection of 110 protein peaks; a few differed significantly between brain tumours and controls (ROC curve; p value).
Conclusions: These techniques can be used for a rapid and efficient method in discovery of serum biomarkers in brain tumours diagnosis.
The advantages could be: less invasive techniques, screening for molecular markers, validation of putative therapeutic targets.

Tuesday 29 June 2010 09:45–17:30

Poster Session
Radiobiology/Radiation Oncology

834 A mouse model for the study of the radiobiology of medulloblastoma

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Medulloblastoma is the most common paediatric malignant brain tumour. The treatment of medulloblastoma involves a combination of surgery, radiation, and chemotherapy. While the overall survival rate for medulloblastoma is relatively good, those patients who experience a relapse of their tumour after initial treatment have a grim prognosis. Because medulloblastoma that relapses post-radiotherapy is nearly universally fatal, our goal is to develop an animal model to better understand tumour relapse in hopes of designing effective therapies. Little is known about the biological response of medulloblastoma to radiation, because clinically relevant doses of radiation are lethal to genetically engineered mouse models. We have developed a mouse model of relapsed medulloblastoma in order to study changes in the tumour after exposure to therapy, and to test novel treatments for their effectiveness after relapse.