

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 controlling 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 tumorigenesis. 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