tumourigenic mechanisms throughout human breast cancer development from various genomic levels in a pathway-based approach.
Material and Methods: Data were collected from six breast cancer cohorts with distinctive clinical parameters to represent the heterogeneity of this disease (approved by the Ethics Committee in Norway). In total, 587 breast tissue samples (20 normal samples, 567 tumour samples from ductal carcinoma in situ to large aggressive breast tumours), with genome-scale DNA copy-number and mRNA expression profiles and with mutation status of selected genes (TP53 and PIK3CA), were investigated.
We identify significant DNA copy-number alterations in different stages of tumour progression; assess the correlation between gene copy number changes and the corresponding mRNA levels; and incorporate the mutation status of selected genes. We integrate these different data types and identify co-occurring and mutually exclusive alteration events, with the potential to distinguish "driver" events from incidental "passenger" events. Combining our findings on the gene-level with known signaling pathway data allows us to identify the molecular processes that drive the different stages of tumour development.
Results: Our study unveils the pathway signatures of human breast tumour progression. We identify candidate chromosomal regions with oncogenic alteration and associated core signaling pathways involved in distinctive stages of tumourigenesis. We also pinpoint the candidate events required for entering subsequent tumour developmental stage. Our analysis confirms that the aberrant alterations in breast cancer tend to occur in a cohesive fashion involving known cancer genes. In addition, new candidate "drivers" in breast cancer progression are also identified.
Conclusions: Alterations of multiple networking genes disrupt critical signaling pathways during breast cancer progression through cooperative mechanisms. The candidate genes identified based on the integrative information from multi-dimensional genomic data are likely to be the "driver" events in breast cancer tumourigenesis.
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824 Deuterium has a key role in tumour development – new target in anticancer drug development

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It is known that the deuterium/hydrogen (D/H) mass ratio is the largest of stable isotopes of the same element, causing differences in the physical and chemical behaviour between the two hydrogen isotopes. Although the concentration of D is more than 10 mM in living organisms the possible role of D had not been investigated for 6 decades after it's discovery in the early 30's.
In order to investigate the possible role of naturally occurring D in living organisms, in cell growth and tumour development, D-depleted water (DDW) was used.
The experiments with DDW revealed that due to D-depletion the cell growth of various cell lines (PC-3, human prostate; MDA, human breast; HT-29, human colon; M14, human melanoma) were inhibited *in vitro*. DDW caused tumour regression in xenotransplanted mice (MDA and MCF-7, human breast; PC-3) and induced apoptosis *in vitro* and *in vivo*. Deuterium depletion inhibited the expression of certain genes (c-myc, H-ras, COX-2) having key role in tumour development.
Breast tumours in 81 dogs and 14 cats showed a response rate higher than 70%; more than 50% of the pets were cured when DDW was used as a single treatment or in combination with surgery.
During the four-month-long DDW administration in the phase II, double blind clinical trial, 7 out of 22 of the prostate cancer patients achieved partial response (PR), while only one patient out of 22 showed PR in the control group (Armitage-test $p = 0.027$, Fisher-test $p = 0.046$). The one year survival was significantly higher in patients treated with DDW (logrank test, $p = 0.029$). The mortality rate decreased substantially in the treated group by the end of the first year (Fisher-test, $p = 0.034$).
The records of 74 women suffering from metastatic breast cancer (MBC) were retrospectively evaluated. Conventional cancer therapy was supplemented with *per os* (PO) DDW treatment, when the daily water intake of the patients was replaced with DDW. The administration of DDW parallel to the conventional treatment produced regression or halted progression in 74.3% of the 74 evaluated MBC patients, increasing the median survival time from the diagnosis of the distant metastasis up to 47.7 months.
We suggest that cells are able to regulate D/H ratio and its changes can trigger molecular mechanisms having key role in cell cycle regulation. The decrease in D-concentration can intervene in the signal transduction pathways thus leading to tumour regression.
We suggest that the recognition of the major importance of naturally occurring D in living organisms can serve as a new target in anticancer drug development.

825 Towards a systems-level view of breast cancer through the joint analysis of multi-dimensional data

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Background: The advent of new technologies has enabled researchers to interrogate genomes at unprecedented resolution, probing their structure and function. However, these data remain largely underutilized due to a lack of scalable methods to detect sparse signals in multi-dimensional datasets. Here we describe the joint analysis of multiple data types to obtain a systems-level view of the genomic architecture of breast cancer in 1000 cases.
Methods: High-density Affymetrix SNP 6.0 arrays were employed to assay allele-specific and total copy number on 1000 fresh frozen tumours and 500 normal samples. Matched RNA from 824 samples was hybridized to Illumina HT-12 arrays for gene-expression analysis. The mutational spectrum of critical cancer loci was surveyed through deep sequencing of a subset of cases. We developed a regularized regression approach to detect aberration hotspots on a genome-wide basis and learn their interaction networks.
Results: Through the integrative analysis of diverse data types, we identified novel breast cancer subtypes with distinct clinical outcomes. We further characterized the genomic landscape of breast cancer in terms of aberration hotspots, ploidy, and preferential allelic amplification. By interrogating alterations at both the DNA and mRNA level in a robust regression framework, we generated a genome wide CIS and TRANS regulatory map of breast cancer. The projection of these events onto pathways yielded a systematic overview of pathway perturbation amongst subtypes, suggesting novel therapeutic targets in patient sub-populations.
Conclusions: The dataset described herein constitutes an invaluable resource to dissect the complexity of cancer. By mining the relationships between multiple data types and associating patient-level data with key clinical variables, we have uncovered new insights into breast cancer biology.

826 Integrated cell cycle and DNA repair signalling network modelling for identification of key molecular regulators in basal-like breast cancer

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Background: Basal-like breast cancer (BLC) is associated with a poor prognosis and there is a lack of targeted therapy to treat it when it fails to respond to first-line chemotherapy. Pathways involved in cell cycle and DNA repair are highly perturbed in BLC, thus facilitating cell survival capacity despite accumulated DNA damage. Cell cycle and DNA repair mechanisms contain a variety of signalling pathways that do not act in isolation, but create molecular networks. Identification of the common molecular regulators will guide the discovery of new strategies to induce synthetic lethality of malignant cells.
Methods: In order to understand in greater detail the orchestration between cell cycle and DNA repair molecular mechanisms, we used a systems biology approach to represent biological processes as comprehensive models based on experimental data retrieved from literature and transcriptomic data on breast tumours. The network is created using the CellDesigner software, which is adapted to further mathematical modelling and studies of signalling network dynamics.
Results: We have constructed an integrated cell cycle and DNA repair molecular signalling network composed of three interconnected layers. The first layer represents core cell cycle pathways and checkpoint proteins. The second layer includes DNA repair pathways related to direct repair, trans-lesion bypass, single strand and double strand DNA repair. The third layer is composed of common regulators and modulator enzymes for cell cycle and DNA repair, such as kinases, phosphatases, etc. that ensure reciprocal influence between cell cycle and DNA repair. We further integrated transcriptomic data from breast tumours into the network and highlighted specific DNA repair pathways, cell cycle checkpoint proteins and common players modified in the disease. To verify the network, we simulated, *in silico*, the familial BRCA1-negative phenotype and inhibition of the base excision repair protein PARP to prove that our model recapitulates some well-described physiological situation.
Conclusions: A comprehensive reconstruction of the cell cycle and DNA repair signalling network allows the integration of multiple crosstalk between

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