of these genomic data have revealed deregulated miRNAs and putative target genes with important functions in bone development and cancers.

### 820 Microarray expression profile of primary human papillary thyroid carcinomas

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**Background:** Thyroid nodules are clinically evident in about 5% of women and 1% of men therefore represent the most common endocrine pathology. Although more than 90% are benign a significant number undergo surgical excision. In 10% of all follicular patterned lesions diagnostic dilemma is presented in a subset of encapsulated lesions with partial nuclear features of papillary thyroid carcinoma and with histological features that fail to place them reliably in either the benign or the malignant category. Microarray gene profiling has shown a promise in the accurate discrimination of benign–malignant discrimination and molecular characterization of thyroid lesions. We focused on not particularly for significantly modulated candidate genes, but for sets of genes acting on similar antiapoptotic and signaling pathways.
**Materials and Methods:** Tumour samples were obtained from 25 patients undergoing thyroid surgery and evaluated on histopathology prior to our experiments. Genomic RNA was isolated from the snap frozen tumour samples of follicular adenomas and sporadic type of papillary carcinomas. We used NimbleGen Human Expression 12X135K Arrays to analyze gene expression alterations between follicular thyroid adenoma and papillary thyroid carcinoma expression profiles. Quantitative RT-PCR and Western blot analysis were done in case of the 10 genes showing the highest expression changes on the array.
**Results:** We found the consequent significant expression regulation of 378 genes 233 of them found to be significantly underexpressed in papillary carcinomas compared to the follicular adenoma tissues. Papillary thyroid carcinomas expressed modulated genes on the NFκB regulatory pathway. Nfκb itself was found to be up-regulated as well as its activator *Med17* and the *Eda1*, the member of the TNF-related ligand family regulating epithelial development, wich has regulatory role in *Nfκ-b*-promoted transcription and *Jnk* signaling. additionally represented constitutive down-regulation *Pparg* and *Mapk, 4, 8* and *10* partaking in NFκB inhibition, and *Cyld1* over expression closely connected to NFκB signaling.
**Conclusions:** Considering the fact that NFkB has already been found to be a promising diagnostic and therapeutic target, our investigation could provide new possibilities for diagnostic, therapeutic and preventative perspectives.

### 821 Dissecting the genetic components of gene expression in breast carcinoma

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**Background:** A series of publications has demonstrated the effects of genetic variation on mRNA expression, and we have demonstrated the association between selected germline variants and gene expression in breast carcinomas. Given the significant role of mRNA expression patterns in breast cancer, we examined to what extent genetic variation from Wide Association Studies may influence expression levels in breast carcinomas.
**Material and Methods:** Genome wide SNP arrays (Illumina 109K) were used to genotype both blood and tumour DNA, and genome wide expression analyses of the tumours were performed (*Agilent 44K*). After normalization, extreme outliers were removed from the expression data. SNPs were filtered on frequency. To address the influence genetic variation in both germline and tumours may have on expression, eQTL analyses were performed in both *CIS* (distance <1 Mbp) and *TRANS* using a linear regression model in R.
**Results:** The *CIS* and *TRANS* eQTL analysis of the germline SNPs resulted in 86 significant hits in 45 different genes after correcting for multiple testing using Bonferroni. We utilized the LogR and BAF information to elucidate the copy number for each allele (A and B). For the total copy number, i.e. A+B, we found 573 BF significant hits corresponding to 318 different probes. The most significant result was seen for a probe within the alkaline phytoceramidase gene *PHCA* on chr11q13.5 (P-value = $4.2\times10^{-33}$), a regulator of cell proliferation and survival. Probes within *ERBB2* were also found to be highly associated with expression in *CIS* (P-value = $8.5\times10^{-26}$). We identified the functional categories of the genes harboring these significant probes by using the Gene Ontology (GO) database, and found that significantly enriched GO categories include hormone biosynthesis. Then studying the allele specific influence on the genome wide expression pattern in the tumour, i.e. A−B, the most significant finding was again seen for a SNP again within the 3′UTR of *PHCA* gene (P-value = $2\times10^{-29}$). Overall we found 86 significant hits corresponding to 81 different SNPs within 33 different genes.
**Discussion:** Our analysis implies the existence of a skewness in breast tumours with respect to what allele that is amplified or deleted, and that their association to variation in expression level may be the driving force behind this selection. These results imply that the germline genetic background may play a significant role in the expression pattern observed in the tumour, as may both total copy number and allele specific aberrations of the tumour.

### 822 Allele-specific aberrations and two dimensional disparity of copy number alterations in breast cancer

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**Background:** Breast cancer is presently one of the most frequent cancer diseases in the world and among women it is the second cause of cancer deaths. Copy number variations (CNVs) are genomic regions differing in copy numbers between genomes. Every diploid has two copies of a locus but in a cancer cell this may vary and leads to occurrence of copy number alterations (CNAs). In this study we focus on the disparity of CNAs in tumour samples compared to blood samples in order to identify directional loss of heterozygosity and chromosomal aberrations. We also report on the overall difference in disparity between stem cell genes compared to non stem cell genes.
**Material and Methods:** We applied a numerical algorithm to Illumina 109K SNPs array data on 112 samples from breast cancer patients. Two outputs of Illumina, B-allele frequency and log R ratio were derived and used to estimate Euclidian distances. For the analysis on disparity in stem cell genes 13 published gene sets were used. Statistical analyses were performed in MATLAB. We applied a filter to remove the non-informative data and divided it into three canonical genotypes AA, AB and BB. For each SNP we compared the genotypes for the samples heterozygous in blood with the genotype corresponding sample in the tumour. We identified SNPs showing preferential disparity from heterozygous towards either the A or B-allele homozygous (horizontal disparity) and towards amplification or deletion (vertical disparity).
**Results and Conclusions:** For the horizontal disparity, 85010 SNPs were included in the analysis after filtering. To identify pathways with a high level of disparity we selected SNPs where 40% or more of the samples were heterozygous (n = 50745) in the blood and again 40% of these showed disparity (n = 5685). From this list we selected SNPs showing a difference in disparity towards AA or BB by 50% or more (n = 172 SNPs representing 160 genes). Using Ingenuity Pathway Analysis the most significantly associated canonical pathways were identified, such as FAK signalling (reported to be required for Ras- and PI3K-Dependent Breast Tumourigenesis). Regarding the analysis of the stem cell genes we see a significantly different level of overall disparity between the stem cell and non-stem cell gene list both in the horizontal and vertical direction (p-value = 0.007166 and 1.370e-09 respectively) as a result of higher level of disparity in the stem cell genes.

## Tuesday 29 June 2010 09:45–17:30

### Poster Session
## Systems Biology

### 823 Pathway signatures in breast cancer progression – a genome-scale study based on integration of biology networks, DNA copy number, gene expression and mutations

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**Background:** Breast cancer is a heterogeneous disease often requiring a complexity of alteration to drive a normal cell towards malignancy and ultimately to a metastatic state. The genetic alterations are most likely reflected by a set of genes or pathways, rather than individual genes. Our high-throughput cancer genomic study is designed to derive the portrait of

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.
We acknowledge Simen Myhre, Jørgen Aarøe, Hans Kristian Moen Vollan for providing the CGH data.

### 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