reduced amount of time, enabling the analysis of million points copy number profiles in a matter of minutes. We applied our new algorithm to a series of 200 Affymetrix SNP 6.0 breast tumour samples.
Conclusions: The CGHseg algorithm is now well suited for high density CGH and SNP array analyses and efficiently detects DNA copy number alterations.

816 FGFR3 mutations in prostate cancer and other tumours

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Background: We previously reported *FGFR3* mutations in prostate carcinoma (PCa). *FGFR3* mutations were associated with low-grade PCa, and also with PCa found in patients with concurrent bladder cancer or skin tumours. The aim of this work has been to further investigate the relationship between *FGFR3* alterations in PCa and the presence of concurrent tumours.
Material and Methods: 41 cases with PCa and other associated tumour types were studied. The PCa series consisted of: 22 incidental (cystoprostatectomy) tumours and 19 clinically significant (biopsy or prostatectomy) cases. Twenty-three PCa were Gleason grade $\leqslant$6, 11 were Gleason grade 7 and 7 were Gleason grade $\geqslant$8. In each case, we studied the PCa and the concurrent tumour (prostate and bladder cancer, n = 32; prostate and skin tumour, n = 6; prostate and colon cancer, n = 2; prostate and lung cancer, n = 1). *FGFR3* exons 7 and 10 were analysed by PCR and direct sequencing.
Results: Eight of 41 (19.5%) PCa presented a mutation in *FGFR3*. From these, 6 were Gleason grade $\leqslant$6, and 2 were Gleason grade 7. Four of 32 (13%) patients with PCa and bladder cancer harboured a *FGFR3* mutation in the PCa, and 5 other cases (16%) in the bladder tumour. In the PCa-skin tumour group, 3 of 6 (50%) PCa presented a *FGFR3* mutation, and other 2 different cases (33%) in the skin tumour. One case harboured *FGFR3* mutations in both tumours, but in different codons. Finally, one case with PCa and colon cancer also had a *FGFR3* mutation in PCa.
Conclusions: *FGFR3* mutations in PCa are associated with an increased frequency of concurrent tumours in other organs, mainly skin and bladder. The lack of coincidence in the presence of *FGFR3* mutations in both the PCa and the associated tumours suggests that they evolve through different pathways.
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817 Integration of gene and miRNA expression profiles in clear cell renal carcinoma cell lines and relationship with VHL gene status

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Clear cell renal cell carcinoma (ccRCC) is the predominant form of kidney cancer, representing 75−80% of primary malignancies of the kidney. The status of the von Hippel-Lindau (*VHL*) tumour suppressor gene, an important regulator of the hypoxia pathway via the hypoxia-inducible factors (HIFs), has been correlated to RCC pathogenesis. *VHL* biallelic inactivation occurs in 80% of sporadic ccRCCs and in all inherited cases, while the remaining 20% harbours wild type gene, thus this molecular heterogeneity needs further investigations. The role of microRNAs (miRNAs) in cancer development and progression is expanding, since a number of evidences suggested that miRNA expression is implicated in tumourigenesis, and miRNAs might function as tumour suppressors and oncogenes in a contest-dependent way.
We used Caki-2 and A498 cell lines as in vitro model of ccRCC pathology, and HK-2 (normal proximal tubular epithelial cell line) as reference sample. We characterized the *VHL* status by direct sequencing and the HIF status by western blot. Affymetrix microarray platforms were applied to assess miRNA profiles (onto GeneChip® miRNA Array, comprising 847 human mature miRNAs) and gene expression profiles (onto GeneChip® Human Gene 1.0 ST Array, including 19,793 annotated genes).
Analysis of differentially expressed miRNAs (DEMs) outlined specific miRNAs in both Caki-2 and A498 that have been found related to ccRCC (e.g. miR-155, miR-21 and miR-221), and in addition some DEMs found only in A498 (*VHL*−/−) that are involved in hypoxia pathway (e.g. miR-210). Functional enrichment analysis showed that some modulated gene (DEG) have a known role in hypoxia and p53 signalling pathways. Additionally, we performed an integrated analysis to combine gene and miRNA expression profiles, under the assumption that, since miRNAs tend to down-regulate their targets, expression profiles of miRNAs and real targets are expected to be anti-correlated.
This integrated analysis exploits miRNAs and target expression information in order to identify most probable functional regulatory interactions occurring in the ccRCC cells, and to reconstruct and study the corresponding post-transcriptional regulatory network. The further integration of these results with DEGs and DEMs will facilitate the elucidation of regulatory circuits important for tumourigenesis and biological processes under relevant post-transcriptional regulation in ccRCC and the interpretation of these results on the basis of *VHL* status.

818 Expression correlations of NFkB signaling and miR146 a/b miR21 and let-7 expression in primary human head and neck squamous cell carcinomas

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Background: The NFkB signal transduction pathway plays as an important link between inflammation and cancer and serves as a promising target for molecular cancer therapy in head and neck squamous cell carcinoma. HNSCCs are characterized by elevated constitutive activity or aberrant regulation of NFkB that acts as a survival factor for malignant cells by its predominantly anti apoptotic function. While the post translation regulation of the NFκB signaling is deep and detailed its post transcriptional regulation is still unclear and there is sparse of data about the expression of the potential miRNA regulators of the NFκB releated genes in primary human HNSCC.
Materials and Methods: Total RNA isolated from fresh frozen primary tumour tissues (n = 10) and formalin fixed paraffin embedded (FFPE) primary tumour tissues (n = 35), fresh frozen non diseased head and neck epithelial tissues (n = 6) and FFPE normal epithelial tissues (n = 8) were analyzed by quantitative real-time PCR for the expression of *Nfκb p65*, *Rel A*, *Ikk1*, *Pparγ*, *Pten*, *Gadd45α*, *Jnk1* and miR146 a/b, miR-21 and let-7.
Results: Significant expression alterations of the investigated genes were found in 92% of the tumour samples. We also found consequent converse and inverse correlation between mRNA and miRNA expressions, especially regarding the *Rel A*, *Pparγ* and the miR-21. Expressions of the fresh frozen samples did not differ significantly from those found in the FFPE samples.
Conclusions: Our data confirm parallel disregulation of miR146 a/b, miR21 and let-7 and their potential mRNA targets in primary human HNSCCs, that could be useful for molecular diagnostics and therapy.

819 Integrated analysis reveals overexpression of miRNA clusters in osteosarcomas

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Introduction: Osteosarcoma (OS) is the most common primary malignant tumour of bone, and almost all most conventional osteosarcomas are high-grade tumours with complex genomic aberrations. Many studies have shown that miRNAs are aberrantly regulated in different human cancers. Elucidating what pathways are affected by a change in miRNA pattern could reveal new avenues for diagnosis or therapy in osteosarcomas.
Material and Methods: We have performed global microarray analysis of a well-characterised panel of 19 OS cell lines, collected within the EU network EuroBoNeT (www.eurobonet.eu), and 4 normal bone samples in addition to mRNA expression data for 71 OS patient samples. Global miRNA expression patterns have been analyzed using the Agilent miRNA array v2.0, mRNA expression patterns using the Illumina HumanWG-6 Expression BeadChip, and DNA copy number changes using the Affymetrix Genome-Wide Human SNP Array 6.0. We used TargetScan 5.1 to predict the most likely targets of the miRNAs and integrated the miRNA and mRNA expression data by calculating the Pearson's Correlation for each of the predicted miRNA-mRNA pairs across all the samples.
Results: We identified 4000 mRNAs that were significantly differentially expressed in OS cell lines compared to bone, of which 40% were confirmed to have the same pattern in the OS patient data. 148 miRNAs were found to separate the OS cell lines from the normal bones. For the target prediction, only conserved miRNA families and conserved target sites with an aggregated P_{CT} value >0.5 were selected. In addition, miRNA-mRNA pairs with low or positive correlation were removed, setting a cut-off at Pearson's correlation <−0.6. This resulted in a set of 38 miRNAs and 119 putative mRNA targets, making up 323 pairs of miRNA-mRNA.
A high number of the miRNAs identified in this study co-localize in clusters in the genome and belong to common miRNA families. These miRNAs have been found to be overexpressed in several solid tumours, but their involvement has not been reported in osteosarcomas. The putative mRNA targets are involved in development and hormone signaling pathways and networks, and have important functions in both bone and cancer.
Conclusions: We have identified miRNAs and mRNAs that are differentially expressed between osteosarcomas and normal bone samples, and integration

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 = 4.2×10^{-33}), a regulator of cell proliferation and survival. Probes within *ERBB2* were also found to be highly associated with expression in *CIS* (P-value = 8.5×10^{-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 = 2×10^{-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