was processed using Nexus Copy Number version 5 (BioDiscovery, Inc., El Segundo, CA) and regions of copy number change were identified using the built-in FASST segmentation algorithm with significance threshold of 1.0E-5. Our sample set contained 237 Primary Tumour samples having therapeutic outcome classified as either Complete Response (n = 130), Partial Response (n = 33), Stable Disease (n = 2), Progressive Disease (n = 15), and Unknown (n = 57). Using a Fisher's Exact test and a maximum p-value of 0.05 we identified significant regions of copy number change between the Complete Response (CR) group and the Progressive Disease (PD) group. These areas included greater loss of 4q13.3−4q35.2, increase in copy number gain for the short arm of chromosome 5 and 6p21.33−6p12.1, in CR vs. PD among other changes. Collectively these regions include 1938 genes and 59 miRNAs. Performing enrichment analysis on these genes, Complement activation, positive regulation of cell-substrate adhesion, and positive regulation of blood vessel endothelial cell migration, are of the highest statistical significance. Taking advantage of mRNA expression data on the same samples, we identified 20 genes that were significantly differentially expressed between the PD and the CR groups from this subset of genes. These 20 genes were then used to cluster the samples into 5 different groups and generate Kaplan-Meir survival analysis showing different degrees of survival for each group with log-rank significance as low as 0.002. This work has allowed us to create a 20 gene signature that is able to predict survival better than just response to therapy.

Reference(s)

[1] Martin-Manso G, Galli S, Ridnour LA, Tsokos M, Wink DA, Roberts DD. Thrombospondin 1 promotes tumour macrophage recruitment and enhances tumour cell cytotoxicity of differentiated U937 cells. Cancer Res 68: 7090–7099, 2008.

794 Allele-specific copy number analysis of breast carcinomas

P. Van Loo[1], S. Nordgard[1], O.C. Lingjærde[2], H.G. Russnes[1], I.H. Rye[1], W. Sun[3], B. Naume[4], C.M. Perou[3], A.L. Børresen-Dale[1], V.N. Kristensen[1]. *[1]Institute for Cancer Research Oslo University Hospital Radiumhospitalet, Department of Genetics, Oslo, Norway, [2]University of Oslo, Department of Informatics, Oslo, Norway, [3]Lineberger Comprehensive Cancer Center University of North Carolina, Department of Genetics, Chapel Hill, USA, [4]Clinic for Cancer and Surgery Oslo University Hospital Radiumhospitalet, The Cancer Clinic, Oslo, Norway*

Background: Whole genome SNP genotyping is an expanding technology to measure genomic aberrations in an allele-specific manner. However, to accurately index all genomic aberrations in a cancer sample, both the ploidy of the cancer cells and the infiltration of non-aberrant cells need to be accounted for in the analysis.
Materials and Methods: We performed genotyping of 112 breast carcinoma samples using Illumina 109K SNP arrays and constructed an algorithm (ASCAT, Allele-Specific Copy number Analysis of Tumours) to estimate the fraction of aberrant cells and the tumour ploidy, and to index all genomic aberrations taking both properties into account. ASCAT allows calculation of "Tumour Profiles" (genome-wide allele-specific copy-number profiles) from which gains, losses, copy-number-neutral events and LOH can accurately be determined.
Results: We present the first allele-specific copy number analysis of the *in vivo* breast cancer genome. Using ASCAT, we obtained Tumour Profiles for 91 of the breast carcinomas (81 %). We observe aneuploidy (>2.7n) in 45% of the cases and an average non-aberrant cell admixture of 49%. By aggregation of Tumour Profiles across our cohort, we obtain genomic frequency distributions of gains and losses, as well as first-time genome-wide views of LOH and copy-number-neutral events in breast cancer. In addition, the Tumour Profiles reveal differences in aberrant tumour cell fraction, ploidy, gains, losses, LOH and copy-number-neutral events between the five previously identified molecular breast cancer subtypes. Basal-like breast carcinomas have a significantly higher frequency of LOH compared to other subtypes, and their Tumour Profiles show large-scale loss of genomic material during tumour development, followed by a whole-genome duplication, resulting in near-triploid genomes. Finally, from the Tumour Profiles, we construct a genome-wide map of allelic skewness in breast cancer, indicating loci where one allele is preferentially lost while the other allele is preferentially gained. We hypothesize that these alternative alleles have a different influence on breast carcinoma development.
Conclusions: We infer tumour ploidy, non-aberrant cell admixture and Tumour Profiles (genome-wide allele-specific copy-number profiles) from genome-wide SNP data of breast cancers and identify specific signatures of aberrations in breast carcinoma and breast carcinoma subtypes.

795 Integrative analysis of genome-wide genetic and epigenetic changes in human osteosarcomas

S.H. Kresse[1], H. Rydbeck[1], A.H. Barragan-Polania[1], R. Duim[2], A.M. Cleton-Jansen[2], O. Myklebost[1], L.A. Meza-Zepeda[1]. *[1]The Norwegian Radium Hospital, Department of Tumour Biology, Oslo, Norway, [2]Leiden University Medical Center, Department of Pathology, Leiden, The Netherlands*

Background: Genetic and epigenetic alterations are frequently seen in cancer, and are responsible for the deregulation of differentiation and proliferation programs. Using different high-throughput technologies, we have analysed genetic and epigenetic changes genome-wide in the well-characterised EuroBoNeT panel of 19 human osteosarcoma cell lines [1]. By integrating different types of genome-wide information, including DNA copy number changes, mRNA expression and promoter DNA methylation, we can identify important genes and transcriptional networks for osteosarcoma development.
Material and Methods: We have analysed the panel of 19 osteosarcoma cell lines, as well as four normal bone samples and two primary osteoblast cultures. DNA copy number changes have been mapped at high resolution using the Affymetrix Genome-Wide Human SNP Array 6.0, methylation of more than 27,500 CpG islands have been analysed using the Illumina Infinium Methylation27 BeadChip, and global gene expression patterns have been obtained using the Illumina HumanWG-6 Expression BeadChip. Data integration is performed using R scripts, and pathway and network analyses are done using GeneGO.
Results: We have identified a number of recurrent regions of DNA copy number changes in the osteosarcoma cell lines, and a comparison between the cell lines and normal bone revealed a number of genes with altered expression and DNA promoter methylation. Integration of the different types of genome-wide data revealed a number of recurrently altered genes involved in important biological functions. DNA copy number, DNA promoter methylation and mRNA expression was further integrated and compared between osteosarcoma cell lines and osteoblast cultures. Further analysis showed a large overlap between the genes identified for each comparison, suggesting multiple types of aberrations in a limited number of critical pathways. Important alterations identified will be validated and further investigated in the EuroBoNeT panel of osteosarcoma patient samples.
Conclusions: Using an integrative approach, we have identified genes, pathways and transcriptional networks frequently altered in human osteosarcomas. These genes may play an important role for osteosarcoma development and will be further investigated.
The EuroBoNeT osteosarcoma cell line panel will serve as a well-characterized genetic and epigenetic model system for basic and preclinical studies.

Reference(s)

[1] Ottaviano et al. Genes Chromosomes and Cancer 2010; 49: 40−51.

796 Molecular diversity in ductal carcinoma in situ (DCIS) and early invasive breast cancer

T. Sørlie[1], A.A. Muggerud[1], M. Hallett[2], H. Johnsen[1], K. Kleivi[1], J. Botling[3], A.L. Børresen-Dale[1], F. Wärnberg[4]. *[1]Oslo University Hospital Radiumhospitalet, Department of Genetics Institute for Cancer Research, Oslo, Norway, [2]McGill University, McGill Centre for Bioinformatics Goodman Cancer Centre, Québec, Canada, [3]Uppsala University Hospital, Department of Genetics and Pathology, Uppsala, Sweden, [4]Uppsala University Hospital, Department of Surgery, Uppsala, Sweden*

Background: Ductal carcinoma in situ (DCIS) is a non-invasive form of breast cancer where cells restricted to the ducts exhibit an atypical phenotype. Some DCIS lesions are believed to rapidly transit to invasive ductal carcinomas (IDCs), while others remain unchanged. Existing classification systems for DCIS fail to identify those lesions that transit to IDC.
Materials and Methods: We studied gene expression patterns related to progression of breast cancer in 31 pure DCIS, 36 pure invasive cancers and 42 cases of mixed diagnosis (invasive cancer with an in situ component) using Agilent Whole Human Genome Oligo Microarrays 44k. Six normal breast tissue samples were also included. qRT-PCR was used for validation. Various analytical methods were used such as hierarchical clustering, Significant Analysis of Microarrays (SAM), Gene Set Enrichment Analysis (GSEA), Database for Annotation, Visualisation and Integrated Discovery (DAVID), and a logistic regression model for gene identification.
Results: All DCIS and invasive samples could be classified into the "intrinsic" molecular subtypes defined for invasive breast cancer. Hierarchical clustering revealed a trend to group by intrinsic subtypes, and not by diagnosis. We observed heterogeneity in the transcriptomes among the DCIS of high histological grade and identified a distinct subgroup containing seven of 31 DCIS with gene expression characteristics more similar to advanced tumours. A set of genes independent of grade, and both ER and HER2 status was identified by logistic regression that univariately classified a sample as belonging to this distinct DCIS subgroup. qRT-PCR of single markers clearly separated this DCIS subgroup from the other DCIS, which consisted of both high and low grade lesions as well as ER- and HER2-positive and negative