Conclusion: Bmi-1 mRNA expression is increased and Mel-18 mRNA expression is decreased in normal breast tissue of cancer patients as compared to normal breast tissue in women having had reduction mammoplasties.

751 Correlation of copy number, gene and protein expression for breast cancer related genes and proteins

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Background: The development of the microarray technology has made it possible to measure multiple entities (eg. genes, probes) simultaneously. For example, spotted DNA probes on microarrays can be utilized to measure gene copy number covering the complete genome. Similarly, whole genome arrays can be utilized to measure the expression of all transcribed mRNA in a sample. At the protein level, the reverse phase protein array (RPPA) can be used to measure and quantify the amount of a specific protein in lysates from multiple samples simultaneously. There are many potential mechanisms of regulation between copy number of a certain genomic region, to the final expressed protein. Methylation, microRNAs, post-translational modification etc. influence the relationship between the copy number level and the expression of the corresponding proteins.
Material and Method: In this study, 267 fresh frozen tumour samples were obtained from high risk breast cancer patients enrolled in the Danish Breast Cancer Cohort 82bc trials and utilized to look at the cis-correlation between copy number, gene expression and protein expression in a panel of proteins selected for their involvement in cancer and the PI3K/AKT-pathway. The Agilent 244 K Comparative Genomic Hybridisation (aCGH) array was utilized for copy number measurements, the Applied Biosystem Whole Genome Array for mRNA expression and classification into molecular subtypes of breast cancer, and the RPPA for protein expression. For the three platforms high quality data for 194, 196 and 210 unique samples was obtained, respectively. The intersection of no. of samples successfully analyzed differed between the three comparisons: copy number–gene expression (154 samples), gene expression–protein expression (164 samples) and copy number–protein expression (161 samples). Spearman correlation was estimated for each measured gene and protein expression across the samples in the three comparisons, and a positive correlation cut-off was set to >0.3. The cut-off reflects significant correlation after Bonferroni-adjustment of the p-values.
Results: We were able to identify entities with a high correlation between all three comparisons (e.g. ERBB2, RPS6KB1, PDPK1). Some genes showed high correlation between copy number and gene expression, (eg. PARP1, RPS6, RB1) but low correlation to the expressed protein, while others showed high correlation between gene and protein expression but low correlation to copy number (e.g. CAV1, CCNB1, ESR1). Finally, some entities showed low correlation across all three platforms (e.g. STK11, FRAP1, SRC).
Conclusion: The correlations for each gene and protein across the three levels of measurements can be utilized to propose drivers of regulation of gene and protein expression, and the correlations vary between different molecular subtypes of breast cancer.

752 Gene expression profile of spontaneously immortalised T lymphocytes

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Background: Immortalisation is a critical step in carcinogenesis. The majority of studies on cell immortalisation have employed models of fibroblasts or epithelial cells, immortalised by virus-transformation or gene-transfection. These procedures introduce unspecific alterations, which make the results obtained in such model systems difficult to interpret. Data on the immortalization of T cells are scarce and inconsistent. Here we used our in vitro model of spontaneously immortalized human T cells to gain insights into the molecular changes responsible for the acquisition of an unlimited growth potential.
Materials and Methods: Three spontaneously immortalised, IL-2-dependent T cell lines, their matched primary lymphoblasts and other primary, IL-2-dependent T lymphoblasts were assessed for gene expression, with the use of the Affymetrix GeneChip Human Genome U133 Plus 2.0 arrays.
Results: Approximately 1300 upregulated and 1100 downregulated geneprobes were found to discriminate between immortalised and primary lymphocytes, the former group comprised GAS1, TP63, IGFBP3, SIX1, IL8 and SYK and the latter – KLF12, BIM, FGL2 and IRF8. Pathway analyses revealed altered cell activation circuits, including T-cell receptor signalling pathway and MAPK pathways, cytoskeletal genes, interferon- and insulin growth factor-related pathways. Among the most significantly changed chromosomal regions there were 2p13−25 and 3q26−29. Significant alterations were also found within the target pathways regulated by 85 transcription factors, including SOX9, YY1, MYC, E2F, AP2, NFAT and NF**K**B.
Conclusions: (1) Immortalisation of T cells involves an immense change in gene expression. This points at the complexity of the processes that drive an unlimited proliferation potential. (2) Overexpression and down-regulation of both tumour suppressor genes and oncogenes, suggest that their actual roles are relative and may depend on the biological context. (3) Spontaneously immortalised T lymphocytes overlap with other immortalisation models in the altered cell activation pathways, but lack the significant changes in the oxidative stress pathway. (4) Chromosome regions 2p13−25 and 3q26−29 of the spontaneously immortalised T lymphocytes host many overexpressed genes. Since amplification of these regions is typical for many cancers, spontaneously immortalised T cells may provide a relevant model to study molecular alterations in these malignancies.

753 Epigenetic regulation of miR-196b expression in gastric cancer

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Background: MicroRNAs (miRNAs) are short noncoding RNAs that play important roles in cellular processes and disease pathogenesis via the control of specific targeted gene expression. The miR-196s miRNA is encoded at three paralogous loci in three HOX clusters and acts as an oncogenic miRNA in cancer progression. The aims of this study were to investigate the mechanisms that underlie the expression and regulation of these three paralogous transcripts of miR-196.
Material and Methods: MiR-196s expression levels in several human cell lines were assessed by real-time PCR approach. The methylation status of miR-196s promoter was analyzed using bisulfite restriction assay, methylation-specific PCR, and bisulfite sequencing in different cells. Luciferase assay studies were carried out to assess the promoter activity of miR-196b promoter in human cell lines.
Results: The methylation status correlated well with miR-196b expression in different cell lines. Treatment with the demethylating drug 5-Aza-dC reactivated miR-196b transcription in methylation-silenced cells. Using in vitro methylation approach, we further provided evidences that promoter hypermethylation tightly repressed miR-196b transcriptional activation in human cancer cell lines. Interestingly, we first demonstrated that the expression of miR-196b was significantly elevated in gastric cancer and that hypomethylation status of miR-196b CpG islands were frequently observed in primary gastric tumours.
Conclusions: Our findings aid in the understanding of miR-196s regulation showing that abnormal DNA hypomethylation to induce overexpression of miR-196b in gastric cancer.

754 DGKa, by regulating atypical PKC, is a key transducer of SDF1a-induced invasive behaviour in breast cancer cells

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Background: Invasive epithelial carcinomashave acquired mesenchymal phenotype, enabling egression from primary tumour site and ability to invade and grow in different tissues, giving rise to distal metastasis. The invasive phenotype, include the ability to produce membrane protrusion in extracellular matrix (ECM) and the ability to degrade it. Chemokines, such as SDF1a, acting through their GPCRs, contribute to the invasive phenotype, sustaining in vitro and in vivo metastatic growth. We previously showed that Src-mediated activation of Diacylglycerol kinase alpha (DGKa) by HGF and VEGF is required for cell scatter and for migration and angiogenesis in epithelial and endothelial cells, respectively. More recently we showed that DGKa, by generating PA, recruits atypical PKCs (aPKCs) in a complex with RhoGDI and Rac, thereby defining the site of Rac activation and ruffle formation (Chianale et al. PNAS 2010). In this study we investigated the role of DGKα in SDF1a-induced invasive and migratory phenpotype of MDA-MB-231 cells.
Material and Methods: Experiments have been carried out in serum starved MDA-MB-231 cells, treated or transfected as indicated and stimulated in presence of SDF1α (50 ng/ml).