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).

Results: We showed that: (i) pertussis toxin sensitive activation of DGKα is required for the invasive phenotype induced by SDF1a in MDA-MB-231 breast cancer cells; (ii) both RNAi silencing of DGKa and pharmacological inhibition of its activity impair Matrigel cell invasion and formatiuon of cell protrusion in ECM; (iii) DGKa mediates SDF1a-induced activation and membrane recruitment of aPKCs of MDA-MB-231 cells; (iv) both DGKa and aPKCs are required for targeting of beta1 integrin and MMP9 at the tip of cell protrusions, and for SDF1a-induced stimulation of MMP9 gelatinolytic activity; (v) expression of constitutively membrane-bound activated form of DGKα in serum starved MDA-MB-231 cells, reproduces membrane protrusion, recruitment of integrin beta1 and MMP9s at protrusion tips and MMP9 activation, even in absence of either SDF1a or other growth factors; (vi) gene profiling of cells expressing myrDGKa indicate that activation of specific pathways mediates its pro-invasive biological activity.
Conclusions: DGKα is an essential requirement for SDF1α-induced breast cancer cell invasion, which regulates an aPKC-dependent pathway, leading to membrane protrusion formation and to targeting and activation of MMP9.

755 MicroRNA-mediated repression of mRNA translation; single nucleotide polymorphisms in microRNA binding sites

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Background: microRNAs (miRNAs) are involved in regulation of gene expression by binding to mRNA target sites and temporarily repress translation or direct mRNA decay. Single nucleotide polymorphisms (SNPs) residing within miRNA binding sites are suggested to alter the affinity between a miRNA and its mRNA target site and thus affect this miRNA-mediated repression of expression[1]. miRNAs have been found to play important roles in cellular processes like differentiation, proliferation, apoptosis and stress response[2] and skewed miRNA-mediated repression of protein expression may lead to diseases like cancer, diabetes and obesity among others.
Material and Methods: miRNA binding sites with a residing SNP was *in silico* predicted and a binding affinity score for each binding site allele computed. We chose three mRNAs, Lass6 (Longevity assurance genes (LAG1) homolog ceramide synthase 6), PTPRJ (protein tyrosine phosphatase receptor type J) and MCC (mutated in colon cancer) with the predicted miR-505, miR-34b and miR-34a binding sites harboring the SNPs rs8304T>G, rs2227947C>T and rs2227947C>T, respectively, for functional validation by Western blot analysis and Luciferase reporter assay technology in the human breast cancer cell line MCF-7. In both methods the experimentally validated miR-101 mediated repression of *EZH2* was used as control.
Results: The Western blot analysis indicates that the protein expression of Lass6 is not affected by an increased concentration of miR-505 in MCF-7 cells, while miR-34b and miR-34a may mediate repression of PTPRJ and MCC expression, respectively. Preliminary analysis of the putative differences in the affinity between the PTPRJ rs2270992T>C alleles of the miRNA binding site of miR-34b indicates that the miRNA mediated repression differs with 62%. For the miR-34a binding site in MCC, which harbors the SNP rs2227947C>T, the Luciferase reporter assay experiments suggest a 15% difference in the efficiency of repression between the SNP alleles.
Conclusions: The miRNAs are involved in regulation of gene expression, and the binding affinity between miRNA and mRNA may be affected by SNPs residing in the miRNA binding sites.

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756 Cancer-related miRNAs like let-7 and miR-21 are already differentially expressed in benign tumours

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Background: Given that women with fibroadenomas are at increased risk of developing breast cancer and that there are genes that are differentially expressed between benign and malignant lesions, we hypothesized that molecular profiles in fibroadenomas may reflect early changes in regulation leading towards proliferation and malignancy. miRNAs are endogenous non-coding RNAs, which play an essential role in the regulation of gene expression. By the use of miRNA microarray technology, we demonstrate that benign tumours are more similar to cancerous tissue than to normal tissue from reduction mammoplastic when considering the miRNA expression profile.
Materials and Methods: miRNA was isolated from 22 biopsies from women with benign breast tumours (fibroadenomas/fibroadenomatosis), 13 samples of malignant breast tumour tissue and 30 samples of normal breast tissue in order to perform miRNA microarray analysis. MiRNA expression profiling was performed by using microarrays containing probes for 866 human and 89 human viral microRNAs from the Sanger database v12.0. Processed slides were scanned and microarray data analysis was performed using Agilent Feature Extraction (FE) Software version 10.7.1.1. For statistical analysis, J-express 2009 software was used to identify differentially expressed miRNAs.
Results: Unsupervised hierarchical clustering using expression information for 322 miRNAs produced 3 major clusters that separated the three different tissue types. A subsequent three class Significance Analysis of Microarrays (SAM) analysis identified 81 miRNAs (101 probes) that are differentially expressed between benign, malignant and normal tissue. Amongst the miRNAs that are the most differentially expressed are members of the let-7 family, miR-21, miR-125b, miR-145, miR-155, and members of the miR-200 family (miR-200b, miR-200c, and miR-141). These miRNAs have previously identified to be tumourigenic and promote tumour growth in different types of cancer, including breast cancer. The same miRNAs are also found to be similarly expressed in both malignant and benign tumours and are most differently expressed from normal tissue. Amongst the miRNAs that are similarly expressed in benign tumours and malignant tumours are miR-21 and let-7. Let-7 targets several tumour suppressor genes while miR-21 targets oncogenes, amongst them is the oncogene RAS which is found to be deregulated in many human cancers. Both miR-21 and let-7 have strong tumourigenic potential and deregulation in these miRNAs leads to deregulation of their target genes that might lead to human cancer.
Conclusion: Benign tumours contain some miRNAs with the same expression level as in malignant tumours. The finding of oncogenic miRNAs such as let-7 and miR-21 in benign tumours indicates that these miRNA may be potential diagnosis biomarkers and probable factors involved in the pathogenesis of breast cancer.

757 Withdrawn

758 Transformation related genes upregulated by c-Jun in highly invasive fibrosarcoma cells

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Background: The expression of an oncogenic transcription factor c-Jun, present as a major component of the activator protein 1 (AP-1) complex, has been found to be constitutively increased in many human cancers and transformed cell lines, such as the highly invasive S-adenosylmethionine decarboxylase-overexpressing mouse fibroblasts (Amdc cells). The aim of this study was to examine the c-Jun-regulated gene expression changes relevant for malignant cell transformation and invasion in Amdc cells.
Materials and Methods: Amdc cells were transfected with a tetracycline-inducible expression system of TAM67 (a dominant-negative mutant of c-Jun lacking the transactivation domain). DNA microarray analysis was used to study differencies in gene expression between Amdc cells inhibited or not in c-Jun expression by TAM67. The identified molecules were functionally characterized by blocking their function in adhesion assays and 3D-Matrigel assays. In addition, immunohistochemical analyses were performed on human fibrosarcomas and the other soft tissue sarcomas.
Results: Only surprisingly few transformation- and c-Jun-relevant genes were found. Among these were integrin subunits α6 and β7, cathepsin L and thymosin β4, all upregulated in Amdc cells and downregulated when c-Jun was inhibited by TAM67. Here, the role of integrin α6 was examined in more detail. As integrins are heterodimeric cell surface receptors, the partner of integrin subunit α6 was first studied, and integrin β1 was found to be the predominant one. By blocking of integrin α6β1 function with specific antibodies, adhesion of Amdc cells to laminin was prevented and cellular invasion fully blocked in 3D-Matrigel. Immunohistochemical analyses showed that immunoreactivity of activated c-Jun correlated with integrin α6 elevation at the invasion fronts of the high-grade sarcomas.
Conclusion: c-Jun has an important role in regulating the molecules involved in cell adhesion and tumour cell invasion, such as integrin α6. As c-Jun has been found to regulate also other steps of transformation, it might be a good target for cancer therapeutic trials.

759 Tumoural growth evolution induces different muscle protein degradation

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Background: The cachectic tumoural growth is able to waste the host tissue, mainly the lean body mass [1]. The systems involved in muscle waste in cachexia are the ubiquitin-proteasome, lysosomal and calcium dependent pathways [1+2].