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.

Reference(s)

[1] Landi D, Gemignani F, Barale R, Landi S: A Catalog of Polymorphisms Falling in MicroRNA-Binding Regions of Cancer Genes. *DNA Cell Biol* 2007
[2] Yang Z, Wu J: MicroRNAs and regenerative medicine. *DNA Cell Biol* 2007, 26:257–264.

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].