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].

Material and Methods: This work analyzed how the tumour evolution could interfere on the proteolytic enzymes activities and the muscle level of 20S subunit (ubiquitin-proteasome pathway), therefore, we investigated the muscle protein degradation in adults rats bearing Walker 256 carcinoma, under different tumour growth conditions. Wistar rats were distributed into 3 groups: **CA** – control group; **IpA** – intraperitoneal tumour-implant; **ScA** – subcutaneous tumour-implant.
Results: The tumoural evolution showed that the **IpA** group survived only 7 days indicating that the tumoural growth is faster than the **ScA**, which survived longer (12−20 days). The body weight was decreased around 11% in both tumour groups. The spleen relative weight was increased especially in **ScA** (5.9% **IpA** and 129.5% **ScA**) while the adrenal relative weight was increased especially in **IpA** (57.6% **IpA** and 5.0% **ScA**). The gastrocnemius muscle weight decreased especially in **ScA** (2.5% **IpA** and 18.8% **ScA**) as well as the protein content (2.2% **IpA** and 8.5% **ScA**), parallel the muscle chymotrypsin-like activity (proteasome system) increased only in **ScA** (64.7%), while muscle lysosomal enzyme (cathepsin B) decreased around 75% in both groups; and the calcium dependent protease (calpain activity) remained unchanged. The 20S subunits expression enhanced in both groups (45% in **IpA** and 64% in **ScA**).
Conclusions: In both tumour-bearing groups, the muscle protein waste involved mainly the ubiquitin-proteasome activity (higher 20S expression), although the **ScA** group presented a higher decrease on muscle weight and protein content suggesting that the participative activity of proteasome-system could be the principal process rather than the other tumoural effects produced in intraperitoneal tumour-bearing rats.

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760 Relationship between NF-kappaB and different clinical-pathological standard factors

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Background: Breast cancer is the second leading cause of cancer related deaths among females worldwide. Actually the search for new markers which could improve individual treatments is one of the most important aims in breast cancer. The NF-kappaB transcription factor family seems to play a pivotal role in breast cancer progression and resistance to chemotherapy. The NF-kappaB family is composed by five subunits (p50, p65, p52, c-Rel and relB) which are inactived in the cytoplasm. When a cell receives any of a multitude of extracellular signals rapidly the subunits form a heterodimer or homodimer which enters to the nucleus and active gene expression. The aim of this study is to analyze each subunit of NF-KappaB and their influence in clinical evolution.
Material and Methods: We analyze frozen tumour samples from 400 patients by hematoxylin and eosin stain and we chose tissues with a tumour fraction higher than 50%. Later we determine the expression of the subunits p50, p65, p52, c-Rel and Rel-B of NF-kappaB by Western Blot and union of NF-KappaB to DNA by ELISA. We characterize the relationship between the different subunits of NF-kappaB and clinical-pathological standard factors.
Results: We have observed that higher activation of p50 is related with presence of the disease in patients who are diagnosing before 50 years old. At the same time we have found p50, p65 and p52 are increased in tumoural tissue against non-tumoural tissue. Also our preliminary data suggest strong relationship between the decrease of at least subunits of p65, p50 and p52 when neoadjuvant therapy is given.
Conclusions: Our preliminary data suggest that higher activation of the different subunits of NF-kappaB is related with higher malignancy in breast cancer and that neoadjuvant therapy could act inhibiting NF-kappaB.

761 Reverse-phase protein arrays as a tool to discover mutation-associated alterations in cell signaling pathways

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Cancer is a result of an accumulation of various factors promoting tumour growth and metastasis. Somatic mutations are major molecular determinants underlying tumour development and progression. However, the complex patterns of genetic alterations within tumours provide a considerable challenge to the understanding of changes in cell signaling pathways that promote cell growth and proliferation.
The effect of known gene mutations on the complex signaling network can be analyzed in a cellular system by using applications for quantitative proteomics. A site-specific recombination system for rapid generation of highly standardized, isogenic cancer cell lines was established to generate a library of cell lines stably over expressing mutated genes. After induction of gene expression, cell viability was determined and the corresponding protein lysates were analyzed by reverse-phase protein array (RPPA) technology. This technique offers an excellent possibility to trace the differences in cell signaling pathways between normal and breast cancer cells. It allows for studying key proteins and their phosphorylation status in several hundreds of samples in parallel. For quantitative protein measurements by RPPA, a protein lysate (i.e. the equivalent of the protein content of few cells) from cell cultures or tissues is immobilized directly on a surface layer of a coated microscope slide. Specific primary antibodies are then used to detect the proteins of interest. A secondary, near-infrared-labeled antibody binds to the primary antibody and signals can be detected using an appropriate scanner.
A cell viability assay served as a selection tool to identify potential candidate proteins and led to a group of key signaling components (i.e. PI3K, PTEN, KRAS, HRAS and B-Raf) which were chosen for time course experiments. Abnormalities in protein expression were detected by RPPA and gave novel insights into cell signal transduction pathways in breast cancer cells.
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762 PALB2: a new inactivating mutation in a breast cancer family

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Background: *PALB2* (partner and localizer of BRCA2) encodes a protein implicated in BRCA2 nuclear localization and stability. Biallelic PALB2 mutations are responsible for N-subgroup of Fanconi Anemia. Monoallelic mutation in *PALB2* are rare and confer an intermediate risk of breast cancer. To further investigate if monoallelic PALB2 mutations confer susceptibility to breast cancer we have sequenced the gene in 95 individuals with familial breast cancer tested negative for BRCA1/2mutations.
Results: The mutational analysis of PALB2 gene identified a frameshift mutation (c.1517delG) that generates a premature stop codon (L451X). The proband was diagnosed at 52 year of age with an infiltrating ductal breast carcinoma of grade 3, expressing both Estrogen Receptor (ER) and Progesterone Receptor (PgR). The mother was also affected by breast cancer at 39 years of age and one out of 3 proband's sisters was affected by basocellular carcinoma and addominal melanoma. To verify the inactivation of the wt allele on proband tumour tissue we performed LOH analysis and we did not find any LOH event. This mutation was also identified in two proband's sisters. One was healthy at 48 years and the other was affected by melanoma at 51 years and basocellular carcinoma at 56 years. We identified 7 missense variants in seven different patients: 5 previously described (T1100T; Q559R; E672Q; P864S; G998E) (frequency >1%), and 2 not previously described (Y334C;L1143H) (frequency respectively 0.5% and 1%). Two variants in the 5′ UTR (−159 G>C; −47 G>A) and an intronic variant (IVS3−57 A>C) have been also identified. The two novel missense variants were tested in 50 healthy controls and the Y334C was found once (frequency 1%). The pathogenicity of these variants is analyzed with two software: SIFT and PolyPhen and these variants result both tolerated for SIFT while for PolyPhen the L1143H results "possibly damaging".
Conclusions: The frameshift mutation c.1517delG generates a truncated protein lacking the WD40 domain essential for the interaction BRCA2/PALB2. Preliminary results indicate that the mutated allele is expressed in peripheral blood lymphocyte of the patient. If coimmunoprecipitation experiments will allow to demonstrate the lack of interaction between the 2 proteins we will add evidence that the interaction between them is essential for BRCA2 function in DNA Double Strand Break Repair.

763 Altered partitioning of the EAG1 potassium channel in the plasma membrane of cancer cells

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Background: The role of the voltage-gated potassium channel EAG1 in the genesis of several tumours has been shown in the last decade. We are interested in determining the distribution pattern and interacting partners of EAG1 within the plasma membrane domains of cells that express EAG1 under physiological conditions (brain) and compare it with their pathological counterpart (tumours).