**310** POSTER

**MALDI-TOF serum protein profiling for the detection of breast cancer using independent validation**

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**Background:** With a lifetime risk currently estimated 1 in 9, breast cancer is among the most common diagnosed malignancies in women. Proteomic expression profiling generated by mass spectrometry has been suggested as a potential tool for the early diagnosis of cancer. The objective of our study was to assess and validate the feasibility of this approach for the detection of breast cancer.
**Methods:** In a randomized block design pre-operative serum samples obtained from 63 breast cancer patients and 73 controls were used to generate high-resolution MALDI-TOF protein profiles as a calibration set. The median age of the patient and control group was respectively, 52 (20−81) and 57 years (39−87). The MALDI-TOF spectra generated using WCX magnetic beads assisted mass spectrometry (Ultraflex) were smoothed, binned and normalized after baseline correction. After pre-processing of the spectra, linear discriminant analysis with double cross-validation, based on principal component analysis, was used to classify the protein profiles. Consequently, the classifier constructed on the first 2 plates was applied on the spectra of an independent validation set. This validation set consisted of serum samples from 29 breast cancer patients and 38 controls. The median age was 59 years (26−87) and 57 years (24−71) for the patient and control group respectively.
**Results:** Double cross-validatory analysis carried out on the protein spectra of the calibration set yielded a total recognition rate of 86%, a sensitivity of 88% and a specificity of 84% for the detection of breast cancer within the calibration set. The AUC of this classifier was 90.3%. When this classifier was applied on the spectra of the independent validation set a total recognition rate of 80.9%, a sensitivity of 72% and a specificity of 89% were found.
**Conclusions:** The use of a randomized block design, but mainly an independent validation set proves that discriminating protein profiles can be detected between breast cancer patients and healthy controls. The high sensitivity and specificity indicate that serum protein profiles could be an promising option for the detection of breast cancer.

**311** POSTER

**Early discovery of genomic correlates of drug response by high-resolution gene copy number profiling**

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Cancer is a highly heterogeneous disease in terms of the genetic profile and the response to therapeutics. An early identification of a predictive genomic marker in drug discovery may help select patients that would respond to the drug in clinical trials. Here we outline our strategy for early identification of genomic correlates of response, which utilizes high-resolution comparative genomic hybridization (CGH) coupled with drug sensitivity screening. The resolution and reproducibility of CGH is optimized by using high-density genotyping arrays that interrogate 114K SNPs distributed across the genome at a median distance of 24 kb. The data are analyzed using an internally developed bioinformatics software package that enables identification of gene copy number changes correlating with a pre-defined class label (drug sensitivity/resistance). Additionally, the software also permits identification of recurrent aberrations and produces complete information on all genes residing within the region of aberration. This, together with convenient visualization features, facilitates target discovery.
We applied this methodology to discover genomic correlates of sensitivity to a novel small-molecule antagonist of Bcl-2 family proteins recently discovered at Abbott Laboratories and shown to induce tumor regression in mouse models. The compound, ABT-737, was tested in a panel of small-cell lung carcinoma, leukemia, and lymphoma cell lines and revealed differential inhibition in each of the cancer types (10 nM mM). The CGH screen of these lines followed by Fisher's exact test analysis revealed a previously unknown amplification on 18q21−22 that was associated with the sensitivity of SCLC cell lines to Bcl-2 antagonists. The region of gain contained several genes known to be critical mediators of apoptosis. Expression microarray profiling showed that the genes residing in the amplified region of 18q are also overexpressed in the sensitive lines relative to the resistant ones. Analysis of SCLC tissue microarrays by FISH revealed that the gain of 18q21.22 is a frequent event in SCLC. Thus, our findings suggest that 18q21−22 copy number will be a clinically relevant predictor for sensitivity of SCLC to Bcl-2 family inhibitors. This genomic marker may have a broader application in cancer, as it leads to overexpression of genes associated with apoptosis evasion and chemoresistance. Taken together, our data demonstrate the potential value of high-resolution CGH for identification of genomic biomarkers.

**312** POSTER

**Proteomic profiling of invasive cancer cells reveals a novel prognostic marker for human breast cancer**

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Unlocking the mysteries of metastasis, a major cause of cancer mortality, is essential for the development of novel therapies and diagnostic methods. Cell surface constituents are the key players in interactions needed for a metastatic dissemination of cancer. We are using subclones of the MDA-MB-435 human carcinoma cell line as a model for the metastatic spread of cancer. One clone metastasizes consistently to the lungs whereas the other fails to grow at any distal site in athymic mice. To study the metastatic process we biotinylated the cell surface proteins and isolated them from the post-nuclear supernatant with magnetic streptavidin beads. Isolated proteins were analyzed with 2D gel electrophoresis and mass spectrometry. Galectin-3 was found to be expressed only on the surface of the metastatic cell line. The expression level of this protein is known to correlate with cancer aggressiveness and metastasis. MHC class I antigen, on the other hand, was down-regulated in the metastatic cell line compared to the non-metastatic cell line. This down-regulation is thought to be associated with tumour invasion and development. We have also identified novel cell surface proteins that could contribute to the formation of distant metastases. In addition, we have discovered two novel splice variants of one of the novel cell surface proteins. These splice variants were expressed as GFP-fusion proteins in order to follow their intracellular localization and effect on cell proliferation, invasive growth and tumour formation in mice. The expression of these splice variants affected the localization of the endogenous protein. In addition, we have analysed the localization and levels of this protein in the array of human breast carcinoma samples (n = 350). This analysis revealed a significant correlation between the expression and localization of our protein and the survival of the patients indicating this protein as a novel prognostic marker for human breast cancer.

**313** POSTER

**Comparison of molecular determinants of angiogenesis and lymphangiogenesis in lymph node metastases and in primary tumours of patients with breast cancer**

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**Background:** Angiogenesis and lymphangiogenesis are complex processes, driven by multiple factors. In primary breast tumours (PTs), VEGFA, -C and -D are the most important (lymph)angiogenic factors. Recently, the induction of lymphangiogenesis in axillary lymph node (LN) metastases of patients with breast cancer was described. The aim of this study was to compare the molecular determinants of (lymph)angiogenesis in LN metastases and in PTs of patients with breast cancer.
**Materials and Methods:** RNA was isolated from FFPE tissue sections of a metastatically involved and uninvolved LN and the PT of 26 lymph node positive patients. By qRT-PCR, the expression of 12 (lymph)angiogenic markers was measured. The expression was correlated with tumour cell proliferation, angiogenesis and lymphangiogenesis, quantified by tumour cell proliferation fraction (TCP%) and (lymphatic) endothelial cell proliferation fraction [(L)ECP%]. TCP%, ECP% and LECP% were assessed on IHC double-stains for CD34/Ki-67 and D2−40/Ki-67, respectively.
**Results:** In involved LNs, the relative gene expression levels of PROX1 ($p < 0.001$) and FGF2 ($p = 0.008$) were decreased and the expression levels of VEGFA ($p = 0.01$) and PDGFB ($p = 0.002$) were increased compared to uninvolved LNs. The expression of most markers was increased in PTs compared to involved LNs. In metastatically involved LNs, the expression of VEGFA correlated with ECP% ($r = 0.54$, $p = 0.009$) and LECP% ($r = 0.76$, $p < 0.001$). In PTs, VEGFA correlated only with ECP% ($r = 0.74$, $p < 0.001$).

VEGFD correlated with peritumoural LECP% (r = 0.61, p = 0.001) and with VEGFC (r = 0.78, p < 0.001). Linear regression analysis confirmed the expression of VEGFA as an independent predictor of ECP% in both PTs ($\beta$ = 0.58, p = 0.03) and LN metastases ($\beta$ = 0.90, p = 0.009) and of LECP% ($\beta$ = 0.65, p = 0.09) in LN metastases. The expression of VEGFD ($\beta$ = 0.88, p = 0.03), not of VEGFA independently predicted peritumoural LECP% in PTs.
**Conclusions:** Our results confirm existing data that in PTs angiogenesis and lymphangiogenesis are respectively driven by VEGFA and VEGFD. In LN metastases on the contrary, both processes seems to be driven by VEGFA. Lymphangiogenesis in PTs and in LN metastases might thus be driven by different factors.

**314** POSTER

**Investigation of the potential of human mesenchymal stem cells (hMSC) as vectors for therapeutic gene delivery to breast tumours**

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**Background:** A variety of gene therapy strategies have been developed and evaluated for breast cancer treatment but clinical responses remain poor. Adenoviral vectors have been commonly used for gene therapy studies. One of the major barriers to effective therapeutic results using this system is the induction of an immune response. Targeting the vector to tumour sites is also a major challenge. The use of mesenchymal stem cells (MSCs) as systemic delivery vehicles for therapeutic genes has been proposed as a method to overcome both limitations as a result of their combined ability to home to the tumour site, and evade the host immune response. This study is aimed at investigating homing of human MSCs to breast cancer primary cultures and cell lines in vitro and in vivo, and to identify factors mediating this migration.
**Materials and Methods:** MSC migration in response to breast tumour cells was quantified using Transwell™ inserts. Chemokines produced by the tumour populations were identified using ChemiArray™ or ELISA. The role of specific chemokines in mediating cell migration was determined using blocking antibodies and recombinant standards of the ligands. An animal model of metastatic breast cancer was established using athymic nude mice, followed by an intravenous injection of fluorescently labelled MSCs. At varying timepoints following MSC administration, mice were sacrificed and tumour tissue harvested for detection of engrafted MSCs.
**Results:** There was a significant increase in migration of MSCs in response to all tumour cells examined, including whole primary tumour explants (2−10 fold increase). Tumour cells were shown to secrete a variety of chemokines including GRO, GRO$\alpha$, IL-6 & 8, MCP-1 and SDF-1$\alpha$. Inclusion of antibodies to MCP-1 and SDF-1$\alpha$ in tumour conditioned medium caused a significant decrease (26−52%) in MSC migration. Successful engraftment of fluorescently labelled MSCs was detected in metastatic deposits of breast tumours in nude mice following systemic administration.
**Conclusion:** These promising preliminary results support a potential role for MSCs as vehicles for tumour-targeted delivery of therapeutic agents to breast cancer.

**315** POSTER

**Inhibition of AKT by novel tetracyclic triterpenoids induces cell cycle arrest and triggers apoptosis in human prostate cancer cells**

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**Background:** Akt are serine/threonine kinases, which control pathways involved in cell metabolism, proliferation and apoptosis. Akt play an important role in progression and chemoresistance of prostate cancer. Indeed, loss of the 'phosphatase and tensin homolog deleted on chromosome ten' (PTEN) expression, a phosphatase inhibiting Akt, is associated with aggressive behaviour of prostate cancer.
**Materials and Methods:** We analyzed the expression and function of Akt isozymes in androgen-dependent LNCaP and androgen-independent PC-3 and DU 145 prostate cancer cells.
**Results:** Akt1 and Akt2, the major isoforms expressed, are constitutively active in all three cell lines. Three structurally different Akt inhibitors exerted cytotoxic effect on LNCaP and PC-3 cell lines indicating that the Akt pathway is indispensable for cell viability. Various Boswellia species contain a mixture of mono- and triterpenoids that possess biological activities including antitumor properties. In search for well-tolerated and stable Akt inhibitors, we have isolated several tetracyclic triterpenoids from the oleogum resin of Boswellia carterii and purified them to chemical homogeneity. 3-Keto–tirucallic acid, alpha-acetyl-tirucallic acid and beta-acetyl-tirucallic acid potently inhibited the activities of human recombinant Akt1 and Akt2 in in vitro kinase assays. Similarly, the triterpenoids inhibited Akt activity immunoprecipitated from PC-3 cells, but did not affect the activity of immunoprecipitated IKK. The triterpenoids inhibited the phosphorylation of cellular Akt and glycogen synthase kinase-3beta, whereas extracellular signal-regulated kinase 1/2 phosphorylation was increased. Further, the compounds inhibited nuclear accumulation of p65/relA, androgen receptor, and the expression of the cell cycle regulators cyclin D1 and c-myc, followed by hypophosphorylation of retinoblastoma protein. These events culminated in cell cycle arrest and induction of apoptosis. Similarly, selective downregulation of Akt1, but not Akt2 expression, by siRNA induced marked inhibition of cell proliferation and apoptosis. In addition, the triterpenoids induced inhibition of proliferation and apoptosis in tumors grafted onto chick chorioallantoic membranes.
**Conclusions:** These results suggest that the inhibition of Akt activity is sufficient to trigger apoptosis in prostate cancer cells. Tetracyclic triterpenoids inhibiting Akt might provide a novel approach for the treatment of human prostate cancer.

**316** POSTER

**Oncogenic H-Ras V12 promotes anchorage-independent cytokinesis**

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During carcinogenesis the cell achieves specific characteristics due to critical genetic changes. These changes cause transformation creating a cell with defect cell cycle control and the ability to divide without attachment to extracellular matrix (ECM). Since loss of cell anchorage to ECM induces untransformed cells to arrest in the cell cycle G1-phase this phase has been suggested to possess the major control of cell anchorage to ECM. A second point at which cell anchorage influences cell cycle progression is during cytokinesis. When non-transformed tissue cells are cultured in suspension they become binuclear.
We hypothesized that cancer cells capable of anchorage-independent growth must overcome controls in all anchorage-controlled cell cycle phases. Therefore we investigated the progression of primary human fibroblasts through each cell cycle phase when cultured without anchorage. Cells were synchronized at the start of different cell cycle phases and the cells were cultured either in suspension or attached to ECM followed by analysis of their cell cycle progression.
We show that cell anchorage to extracellular matrix do not control progression through the S and G2 phases in primary human fibroblasts, which also progress through most of mitosis with normal morphology. The cells in suspension initiated cytokinesis by forming midbodies with Aurora B, Rho A and alpha-Tubulin localized as in attached cells. The suspended cells also formed cleavage furrows and initiated but were unable to complete contraction, and instead collapsed and became binuclear. However, Ras-transformed fibroblasts and two cancer cell lines progressed through the entire cell cycle without anchorage to ECM. We therefore suggest that the ability to progress through cytokinesis without anchorage is achieved during carcinogenesis and might be a prerequisite for anchorage-independent growth.

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**APC, CDH1 and CTNN1B promoter CpG islands methylation patterns during ductal breast carcinoma progression**

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**Background:** In spite of earlier detection and better management, mammary tumors are still the primary cause of cancer deaths among women. The advent of mammography screening has led to an increased detection of pre-invasive mammary lesions, and to a better elucidation of the pathological events that precede the development of invasive breast carcinoma. Among the pathogenetic events leading to breast tumorigenesis, CpG island hypermethylation is emerging as one of the main mechanisms for inactivation of cancer related genes. In this study