treatments resulted in tumor radiosensitization while only those associated with an increase in blood flow resulted in chemosensitization. Treatments involving consumption effects did not improve sensitivity to chemotherapy.
Conclusions: It is necessary to evaluate a combination of MR parameters to be predictive in terms of tumor response to treatment. For example, the evaluation of BOLD parameters or DCE-MRI parameters alone could lead to a misinterpretation since a lack of change in perfusion or in BOLD SI are not always associated with a lack of change in oxygenation (and thereby in radiation sensitivity).

P14

Detection of novel biomarkers by plasma proteomic profiling of oesophageal adenocarcinoma mouse xenografts in response to epirubicin, cisplatin and 5-fluorouracil

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Background: Oesophageal cancer is the 9th most common malignancy worldwide with an increasing incidence in recent years. Use of neoadjuvant chemotherapy in locally advanced cancer prior to surgery has been shown to improve outcomes, but the response to therapy is variable and survival rates poor. Hence, the effective use of chemotherapy could be greatly improved by the availability of biomarkers that predict response to therapy. The purpose of this study was to identify candidate biomarkers in mouse xenograft models of oesophageal cancer.
Methods: OE19 (adenocarcinoma) xenografts were established in SCID immune-deficient mice and tumour growth rates recorded. A clinical dose of epirubicin, cisplatin or 5-fluorouracil was administered to xenografts (or controls), by once weekly peritoneal injection for up to 3 weeks. Plasma collected from treated and untreated xenografts and controls was analysed by SELDI-TOF MS using Ciphergen CM10 (weak cationic) and Q10 (strong anionic) protein chips. Protein peaks (m/z) were identified that differed significantly ($p < 0.05$) between the treatment groups for each drug. Samples containing statistically significant markers were fractionated on anion exchange spin columns and approximate pI determined. Searches were performed on the Swiss-Prot database for proteins with the target mass and pI.
Results: Tumour growth was suppressed in treated compared with untreated xenografts. A number protein peaks were identified that differed significantly ($p < 0.05$) between the treatment groups with each drug. Several of these protein peaks were also shown to be common to the three drugs. Determination of the approximate pI of the proteins by anion exchange fractionation has allowed a preliminary identification of two of these peaks.
Conclusions: These experiments have established a response to chemotherapy in oesophageal adenocarcinoma xenografts by proteomic profiling of plasma. A preliminary identification of two markers has been made. Candidate markers are being further identified and will be tested in clinical patients.

P9

Altered expression of plasma membrane proteins on breast cancer cells capable of forming metastasis. Identification by comparative proteomic analysis

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Background: Breast cancers often spread to regional lymph nodes and distal sites such as liver, lung and bone marrow and may appear many years after resection of the primary tumor. The formation of metastasis is a complex multi-step process. One of the steps includes the ability of disseminated cells to establish a secondary tumor at the distant site. The cancer cell proteins involved in this process, and cell surface proteins in particular, are poorly identified. To address this we identified proteins that exhibited altered expression level in a set of isogenic breast cancer cell lines; one cell line being capable of disseminate from the primary tumor by vascular channels and metastasize to distal sites, while the other was equally tumorigenic and able to disseminate single cells to distal organs, but remained dormant and did not metastasize.
Methods: Membrane purification and comparative LC-MS/MS proteomic analysis using 'stable isotope labelling of amino acids in cell culture' (SILAC) in a model system of two isogenic breast cancer cell lines (M-4A4/NM-2C5) derived from the MDA-MB-435 cell line by single cell cloning. Data was validated using protein chemistry methods, immuno-cyto- and -histo-chemistry analysis.
Results: Thirteen proteins were up-regulated while three proteins were down-regulated more than two-fold among more than three hundred validated membrane proteins. Among the cell surface proteins being up-regulated in the metastatic cell line compared to the non-metastatic one we found 5′nucleotidase, Ndrg1, integrin-β1 and MHC class II proteins. The up-regulation of these proteins on the metastatic cell line was validated using flowcytometry, immunocytochemistry and Western blotting. The expression of selected proteins was also examined with immunohistochemistry on breast cancer biopsies of the primary tumor from patients with a known medical history of recurrence status within a ten-year follow-up period.
Conclusions: The cell surface membrane proteins with altered expression level in the metastatic vs. the non-metastatic cell line may bring insight into the initial stages of metastasic development and potentially be clinical attractive for cancer diagnosis or therapy.

P34

Comparison of expression and distribution of eEF1A in normal and cancerous tissue

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Background: The eukaryotic elongation factor 1A is well known for its role in the elongation cycle of protein synthesis, where it catalyses the delivery of aminoacyl tRNA to the ribosome. It is however also believed to function in many other cellular processes including signal transduction, cytoskeletal organization and apoptosis. The protein is believed to play a role in tumorigenesis, as elevated levels of eEF1A has been shown to render rodent fibroblast cells highly susceptible to transformation induced by 3-methylcholanthrene and ultraviolet light, which is likely to make the cells more vulnerable to malignant transformation. Supporting this theory, elevated level of eEF1A mRNA has been found in pancreas, colon, breast, lung and gastric tumors compared to healthy tissue. We have examined whether if there is a correlation between protein expression and distribution of eEF1A, and the development of cancer.
Methods: Using the phage display technology, a recombinant Fab antibody reacting with eEF1A was isolated when searching for human autoantibodies from patients with Felty's syndrome. This Fab fragment called ANA15, is believed to bind a conformational epitope of eEF1A, present in the nucleus of cells. A commercial antibody (CBP-KK1), which was also used, binds to eEF1A in cell cytoplasma. Formalin-fixed paraffin-embedded tissue sections were incubated with diluted lysate of E-coli cells producing ANA15, and bound Fab detected with a goat anti-human Fab. Similarly, sections were incubated with CBP-KK1 and detected with anti-mouse IgG. Double staining with ANA15 and an antibody against Ki-67, which is a cell proliferation marker, was also performed.
Results: Many tumor types (e.g. endometrial and bladder carcinoma) exhibited stronger staining with ANA15 and CBP-KK1 than observed in the corresponding healthy tissue. The majority of colon, endometrial and thyroid carcinomas, however, showed a reduced staining with ANA15 when compared to normal tissue. Likewise colon and endometrial tumors showed decreased CBP-KK1 staining, whereas several thyroid carcinomas showed an increased staining with CBP-KK1. The double staining analysis showed that there was no correlation between the presence of nuclear eEF1A and Ki-67 expression.
Conclusions: The staining patterns varied between the tumor types, and therefore no general connection between the expression eEF1A and cancer was observed. However, differences in expression levels between some of the tumors (e.g. colon carcinoma) and the corresponding normal tissue were observed. No correlation between staining with ANA15 and anti-human Ki−67 was found, indicating that nuclear eEF1A is not only associated with cell cycle progression, but also reflect metabolic activity of the cell. Combining staining for eEF1A and Ki−67 may add valuable information when characterizing cancers.

P81

Targeted quantitation of lung cancer biomarker candidates by liquid chromatography–tandem mass spectrometry with multiple reaction monitoring

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Background: Currently there are no proven molecular strategies for the early detection of lung cancer of established utility in the clinic. In order to identify candidate tumor specific proteomic biomarkers we have employed liquid chromatography-tandem mass spectrometry (LC-MS-MS) with multiple reaction monitoring (MRM) in the tissue first and then in the serum of patients with and without lung cancer.
Methods: In this approach, MRM is used to detect MS-MS fragmentations of specific tryptic peptides derived from the proteins of interest. We used both Thermo LTQ linear ion trap and Thermo TSQ Quantum triple quadrupole instruments to monitor up to 10 (LTQ) and 240 (Quantum) MRM transitions to detect up to 10 (LTQ) and 60 (Quantum) proteins in a single

run. Each peptide is monitored by 4 transitions corresponding to major β- or γ-ion fragmentations. The integrated chromatographic peak areas for the transitions were summed and compared to summed peak areas for beta-actin or human serum albumin, which was used as normalization standards.
Results: We applied this approach to screen lung cancer biomarker candidates in a test set of 20 tissue samples from patients with and without lung cancer. The MRM analyses detected 9 candidate proteins. These candidates were differentially expressed in unfractionated tissue lysates from cases and controls. In addition to these, we have found that prefractionation of protein extracts or peptides derived from protein tryptic digests allows detection of lower abundance candidates.
Conclusions: Current efforts are focused on methodological and analytical refinements to confirm the optimum number of peptides, the number of transitions to monitor, and the applicability of this approach to serum or plasma samples. Our results suggest that this proteomic method may have potential for accurately quantifying candidate lung cancer proteomic biomarkers in complex biological specimens.
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P22

Efficient separation of plasma membrane proteins allowing identification of increased numbers of cell surface markers associated with breast cancer metastasis by comparative quantitative proteomics

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Background: The molecular mechanisms involved in the metastatic process of breast cancer cells are complex and incompletely understood, but cancer cell surface proteins seem to play a pivotal role in several steps. Defining the cell surface proteome in the metastatic context is, furthermore, of importance for identification of therapeutic targets. Traditionally, studies of protein expression have been restricted to examination of a small set of proteins, but recent advances in the field of mass spectrometry have enabled simultaneous analysis of large numbers of proteins in complex mixtures.
In this study we have examined two isogenic breast cancer cell lines, equally tumourigenic in nude mice, but exhibiting diametrically opposite metastatic capabilities. We developed efficient methods for isolation of cell surface proteins and analyzed these by comparative, quantitative mass spectrometry (MS) thereby identifying cell surface markers with altered expression pattern on metastatic vs. non-metastatic breast cancer cells.
Methods: The proteome of the metastatic cell line was metabolically labeled with C13 arginine and lysine by SILAC (stable isotope labeling by amino acids in cell culture). Cells from both cell lines were mixed in a 1:1 ratio and a crude membrane protein fraction isolated. The membranes and the proteins embedded herein were separated by Percoll/sucrose density gradient and fractions enriched in cell surface proteins and with little mitochondrial contamination are identified by enzymatic assays.
The cell surface proteins were enzymaticly digested and analyzed by LC-MS/MS. The proteins were identified and quantified by the VEMS 3.0 software.
Results: By using dual isotopic labels as compared to a single label the number of identified proteins that could be quantified were increased from less than 50% to more than 90%. As cell surface proteins generally are low abundant the percentage of identified proteins that were membrane proteins could be increased to 60% by analyzing each sample four times by LC-MS/MS. Using this method we have identified more than 1000 different proteins. Thirteen cell surface proteins have been identified as potential markers of metastatic breast cancer.
Conclusions: Inclusion of a Percoll/sucrose gradient provides an efficient mean of isolation of cell surface proteins with little contamination from other cellular compartments. Combined with the metastatic cell model, SILAC, and LC-MS/MS this protocol identifies potential targets for future drug development.

P4

Genes for normalization of qRT-PCR data in breast cancer

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Background: Quantitative real-time RT-PCR (qRT-PCR) has become a valuable molecular technique in basic and translational biomedical research, including cancer, and is about to become useful for clinical testing. To relate the obtained values between samples, the data needs to be normalized. This can be done in various ways; the most accepted being to internal, stably expressed, reference genes. Recently the traditionally used reference gene GAPDH has been shown to be influenced by the hormone oestradiol, while B2M may be influenced by factors present in brain tissue of alcoholics, emphasizing the need to identify the optimal genes to be used for normalization, within the tissue to be analyzed.
Methods: In this study we identified genes to be used for normalization of qRT-PCR data for estrogen receptor positive (ER+) invasive breast cancer (IBC) and also examined their applicability for ER− IBC, normal breast tissue and breast cancer cell lines. The reference genes investigated were RPLP0, TBP, PUM1, ACTB, GUS-B, ABL1, GAPDH and B2M, as well as the cytokeratin genes KRT14, KRT18 and KRT19.
Biopsies of 11 surgically removed ER+ IBCs, 4 ER− IBCs, 3 normal breast tissues and 3 ER+ cell lines were examined and the data analyzed by descriptive statistics, geNorm and NormFinder. In addition, the expression of selected reference genes in laser capture microdissected ER+ IBC cells, were compared with that of whole-tissue.
Results: TBP, RPLP0, PUM1 and ACTB were identified as the most suited for normalization of qRT-PCR data of ER+ IBC samples, as both geNorm and NormFinder consented on these. Further, TBP, RPLP0 and PUM1 were also identified by both programs for the collected group of human samples (ER+ and ER− BC and normal breast tissue).
Conclusions: In conclusion, these genes should be the reference genes of choice when performing qRT-PCR on normal and malignant breast specimens.

P30

Biological role of NHERF1 protein in breast cancer

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Background: NHERF1 is a PDZ domain containing protein that recruits membrane receptors/transporters and cytoplasmic signalling proteins into functional complexes. NHERF1 expression is altered in breast cancer but its effective role in mammary carcinogenesis remains undefined. We reported (Cardone RA, 2007) that NHERF1 overexpression in breast cancer is associated with invasion and aggressiveness of the disease. To further understand NHERF1 function and its biologic role in breast cancer, we analyzed NHERF1 protein expression in breast cancer patients.
Methods: Immunohistochemistry for NHERF1 was performed using EBP50 rabbit polyclonal antibody in 61 breast cancer patients. In particular, we examined 22 primary tumours from node negative (N0) patients, 19 primary tumours and metastatic lymph node from patients without distant metastasis (N1M0); 10 primary tumours together with metastatic lymph node and metastases from patients with distant metastasis (N1M1) and 10 carcinoma in situ (CIS). Moreover, NHERF1 protein expression was also evaluated in all normal tissue surrounding breast cancer. Colocalization of NHERF1 and HER-2neu was also investigated on high HER-2neu expression tumour tissues. Immunohistofluorescence for NHERF1 (polyclonal) and HER-2neu (monoclonal) was performed using the Alexa 488 goat anti-mouse IgG1 and 568 goat anti-rabbit IgG.
Results: NHERF1 positivity was present as membranous staining, especially at the luminal aspects of cells in normal epithelia, and as diffuse cytoplasmic staining in tumour and metastatic tissues. Interestingly, protein localization is strictly limited to the apical membrane region of the normal lobules, also when they are present in tumoral tissues.
A significantly higher NHERF1 cytoplasmic-expression and a lower protein membrane-expression have been found in tumour tissue with respect to normal ($p < 0.001$). Furthermore, NHERF1 cytoplasmic expression was higher in lymph node tissues with respect to normal ($p < 0.001$), while no difference was observed between tumour and metastatic tissues. These results have been confirmed in the different subgroups of patients.
Conclusions: Our study on human breast cancer tissues suggests that breast carcinogenesis is characterized by a different subcellular localization of NHERF1 protein from membrane to cytoplasm perhaps due to different binding with cell membrane. Ongoing immunohistofluorescence and confocal studies will further analyze the colocalization of NHERF1 and other target proteins.

P3

Randomised phase III clinical trial to evaluate the efficacy and safety of an integrated treatment (diet, pharmaco-nutrional and pharmacological) in cancer patients with cancer-related anorexia/cachexia and oxidative stress: interim results

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Background: In April 2005 a phase III randomised study was started to establish which was the most effective and safest treatment of cancer-associated anorexia/cachexia syndrome (CACS/OS) able to improve identified primary endpoints: increase of lean body mass (LBM), decrease