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