modify plasma proteomic expression profile during tumor development. The aim of this study is to explore the application of plasma matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) proteomic patterns to distinguish prostate cancer (PCa) patients from healthy individuals.
Materials and Methods: The EDTA plasma samples have been prefractionated using magnetic beads kits functionalized with weak cation exchange coatings. We compiled MS protein profiles for 57 patients with PCa and compared them with profiles from 174 healthy controls. The MALDI-TOF spectra were analyzed statistically using ClinProTools™ bioinformatics software.
Results: In dependence on the sample used up to 378 peaks/spectrum could be detected in a mass range of 1000–20000 Da, 164 of these proteins had statistically differential expression levels between PCa and Compared to controls, 70 peaks are increased in PCa and 94 peaks are decreased. The series of the peaks were automatically chosen as potential biomarker patterns in the training set. They allowed to discriminate plasma samples from healthy control and samples from PCa patients (sensitivity and specificity >92%) in external validation test. Some peptides, included in this combination (for example, with m/z 4965, 5100), potential cancer markers and require additional research.
Conclusion: These results suggest that plasma MALDI-TOF MS protein profiling can distinguish patients with PCa and also from healthy individuals with relatively high sensitivity and specificity, and the MALDI-TOF MS is a potential tool for the screening of prostate cancer.

PP 18

Transglutaminase 2 as an independent prognostic marker for the survival of Korean patients with non-small cell lung cancer

C-M. Choi, S-J. Jang, B-H. Nam, H-R. Kim, S-Y. Kim, K-M. Hong. *Asan Medical Center, Seoul, South Korea*

Background: Expression of transglutaminase 2 (TGase 2) in cancer is related to invasion and resistance to chemotherapeutic agents in several cancers. However, no study has clinically validated TGase 2 as an independent prognostic marker in lung cancer.
Materials and Methods: The significance of TGase 2 expression as an invasive/migratory factor was addressed by in vitro assays employing down-regulation of TGase 2. The significance of TGase 2 expression as a prognostic indicator was assessed by immunohistochemical staining in 429 early-stage Korean non-small cell lung cancer (NSCLC) patients.
Results: TGase 2 expression increased the invasive and migratory properties of NSCLC cells in vitro. TGase 2 was expressed at high levels in A549 cells and was barely detectable in H23 cells. A549 cells exhibited greater invasiveness than H23 cells, suggesting that invasiveness might be related to TGase 2 expression levels in NSCLC. To futher investigate the role of TGase 2, we transiently transfected A549 cells with TGM2 siRNA to knock down TGase 2 levels. Notably, siRNA-mediated TGase 2 knockdown significantly reduced the invasiveness of A549 cells. Knockdown of TGase 2 by siRNA also reduced the migration of A549 and H1299 cells. In the analysis of immunohistochemistry results, Of the 429 NSCLC tissue samples, 93 (21.7%) showed intermediate TGase 2-immunopositivity and 88 (20.5%) showed strong TGase 2-immunopositivity. TGase 2 levels were significantly higher in adenocarcinoma than in squamous cell carcinoma ($p < 0.001$), in females than in males ($p < 0.001$), and in nonsmokers than in smokers ($p < 0.001$). TGase 2 expression in tumors was significantly correlated with recurrence in NSCLC ($p = 0.005$) or in non-adenocarcinoma subtype ($p = 0.031$). Multivariate analysis also showed a significant correlation between strong TGase 2 expression and shorter disease-free survival (DFS) in NSCLC ($p = 0.029$ and HR=1.554), or in non-adenocarcinoma subtype ($p = 0.030$ and HR = 2.184). However, the correlation was not significant in adenocarcinoma subtype.
Conclusion: TGase 2 expression was significantly correlated with recurrence and shorter DFS in NSCLC, especially in non-adenocarcinoma subtype patients, possibly reflecting TGase 2's role in invasion and migration.

PP 35

Evaluation of betaV-tubulin expression as novel predictive biomarker for clinical benefit from treatment with taxanes in non-small cell lung cancer (NSCLC)

D. Christoph, T. Gauler, M. Engelhard, D. Theegarten, C. Poettgen, R. Hepp, M. Schuler, F. Hirsch, W. Eberhardt, J. Wohlschlaeger. *University of Colorado Denver, Department of Medicine, Division of Medical Oncology, Denver, USA*

Background: Taxanes target microtubules composed of αβ-dimers and change their polymerization or depolymerization dynamics, leading to mitotic arrest and cell death. Increased expression of the βIII-tubulin isotype has been inconsistently associated with a poor outcome in NSCLC patients (pts) treated with paclitaxel (PTX). β-tubulin isotypes consist of a family of 8 members with biologically different subfamilies. In particular, the βIII/βV subfamily leads to PTX resistance in vitro and is expressed in cancer cells with inversely proportional patterns of expression (low βIII-/high βV-tubulin and vice versa). We hypothesize that combined βIII/βV-tubulin protein expression may predict outcome and response following PTX treatment.
Materials and Methods: Pretreatment samples from 58 locally advanced or oligometastatic NSCLC pts treated with PTX combined with platinum as an induction treatment (CTX) and followed by radiochemotherapy (RCTX) with vinorelbine and platinum were retrospectively analyzed. βIII/βV-tubulin protein expression levels were evaluated by immunohistochemistry using the H-Scoring system (ranging from 0 to 300), which is determined by the product of intensity of a specific tumor cells immunoreactivity (range 0 to 3) and the percentage of positive tumor cells. Radiographic evaluation of response was performed according to RECIST.
Results: Median pretreatment H-score for βIII was 110 (range: 0–290) and 160 for βV (range: 0–290). Using the log-rank test and the median H-score as cut-off, we found a significant association between improved overall survival (OS) and low βIII-tubulin protein expression (median OS of 2,070 vs 642 days; HR 0.3292, 95% CI 0.1137 to 0.9535; $P = 0.0406$). Surprisingly, prolonged progression-free survival (PFS) was associated with high βV-tubulin protein expression (median PFS of 496 vs 252.5 days; HR 1.961, 95% CI 1.031 to 3.732; $P = 0.0402$). High βV-tubulin protein expression was associated with objective response (OR) (mean H-score 160.3 for CR+PR vs 117.5 for SD+PD pts, $P = 0.0135$) or disease control rate (DCR) to induction CTX (152.4 for CR+PR+SD vs 100.0 for PD pts, $P = 0.0484$), but not for RCTX.
Conclusion: This is the first report of βV-tubulin in NSCLC. Based on our retrospective study, baseline βV-tubulin expression may predict outcome of PTX-based therapy in NSCLC. In contrast to βIII-, βV-tubulin expression is a predictor for OR or DCR to PTX therapy. Confirmation of the prognostic/predictive value of combined βIII/βV-tubulin expression by prospective studies is warranted.

PP 100

Detection of DNA methylation biomarkers in sputum samples for the confirmation of NSCLC

J. Clark, M. Kelly, F. Moreau, J. Bigley, D. Iden, S. Belinsky. *MDx Health, Liege, Belgium*

Background: Lung cancer is the leading cause of cancer-related death among men and women in the United States and Europe with early diagnosis remaining elusive. The use of DNA methylation markers for early detection of lung cancer in sputum has shown promise in multiple clinical studies. The development of an assay to detect aberrantly methylated genes associated with NSCLC in sputum samples would be an advance in the quest for a screening test. The aim of this study was to determine if a set of specifically selected methylated gene markers could discriminate NSCLC from cells collected from patients with compromised pulmonary function but found to be cancer-free.
Materials and Methods: Sputum samples were obtained on three consecutive days from patients with with different stages of NSCLC ($n = 40$) and matched controls ($n = 52$) consisted of symptomatic subjects at risk for lung cancer. DNA from fresh frozen sputum samples were extracted using standard procedures. Methylation status was defined on 11 genes (TAC1, HOXD1, SFRP2, RASSF1A, HOXA9, JAM3, CDO1, SOX17, DPYSL4, GPNMB and GREM1) including the ACTB reference using methylation specific PCR (MSP). Methylated gene copy/ACTB copy ratios were calculated and cut off was defined using scatter plot and ROC curve.
Results: Sensitivity and specificity values obtained from the tested genes ranged between 13 to 43% and 94 to 100% respectively. MSP results obtained from mRASSF1A showed specificity and sensitivity values of respectively 96% and 60%. A combination of RASSF1A with TAC1, GREM1 or HOXA9 resulted in sensitivity between 75 to 80% with specificities between 90 to 96%.
Conclusion: Our results clearly show that DNA methylation biomarkers can be used to confirm the diagnosis of NSCLC in sputum samples. RASSF1A was the best marker in terms of sensitivity and specificity. If we combine RASSF1A with TAC1, GREM1 or HOXA9 we increase the sensitivity without affecting the specificity. Additional studies employing predetermined cutoff values for each gene are planned.

PP 4

Identification of specific biomarkers for glioma-initiating cells and glioma tumors

V. Clément-Schatlo, M. Tenan, B. Schatlo, D. Marino, K. Burkhardt, K. Schaller, I. Radovanovic. *University of Geneva, Geneva, Switzerland*

Background: Brain tumors make up to 2% of all tumors in adults and, in their malignant form (grade IV or glioblastoma (GBM)) remain one of