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

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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)

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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

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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

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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

the most aggressive diseases with a 2-years survival rate of 32% with today's available standard treatments. Few biomarkers (serum and protein) such as GFAP, YKL-40 and S100b can be found in a proportion of human glioma samples yet due to their relative novelty and lack of a solid body of independent studies to date, none of these biomarkers have yet gained wide-spread acceptance in the neuro-oncological community. Importantly, it is unknown whether the cells, which express these markers are tumor-initiating cells or cells from the bulk representing the differentiated part of the tumor.
Materials and Methods: In order to evaluate the relevance of the above mentioned protein markers as specific glioma-initiating cells (GIC) biomarkers and potentially identify new biomarkers, we took advantage of a recently published procedure to discriminate glioma-initiating cells from the non-initiating cells within brain tumors (Clément et al, 2010).
Results: We revealed that known biomarkers such as GFAP, YKL-40 nor S100b showed low or almost no expression in the GIC compartment. We therefore screened for novel and specific biomarkers in purified initiating and non-initiating cell populations. Using Illumina microarrays, we identified 411 candidate genes (criteria: 2 fold-change and $p < 0.05$) and selected 11 candidate biomarker genes for the GIC compartment and 10 candidate biomarker genes for the non-GIC compartment. Using quantitative real-time PCR and immunochemistry/flow cytometry, we validated 2 novel biomarkers for GICs and some for non-GICs at the mRNA and protein levels.
Conclusion: Altogether our results demonstrate that pre-determined/known biomarkers cannot be used as biomarker for glioma-iniating cells. Furthermore, they point out the needs to have a better understanding of the molecular biology of this subpopulation of GICs within the tumor in order to identify and develop novel/specific biomarker for glioma-initiating cells.

PP 5

In vivo metabolic profiling of glioma-initiating cells using proton magnetic resonance spectroscopy at 14.1 Tesla

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Background: In the last decade, evidence has emerged indicating that the growth of a vast majority of tumors including gliomas is sustained by a subpopulation of cancer cells with stem cell properties, called cancer initiating cells. These cells are able to initiate and propagate tumors and constitute only a fraction of all tumor cells.
Materials and Methods: Human glioma cells were injected into the striatum of nude mice (Clement et al, 2010). These mice were examined on days 7, 14, 21, 28 and 35 after the cell injection on a 14.1 Tesla animal MR scanner (Mlynárik V et al. 2006; Gruetter R, 1993). Metabolite concentrations were estimated from the spectra using LCModel.
Results: We showed that intracerebral injection of cultured glioma-initiating cells (CGICs) into nude mice produced fast-growing tumors showing necrosis and gadolinium enhancement in MR images, whereas gliomas produced by injecting freshly purified glioma-initiating cells (FGICs) grew slowly and showed no necrosis and very little gadolinium enhancement. Using proton localized spectroscopy at 14.1 Tesla, a decrease of N-acetylaspartate, glutamate and glucose concentrations and an increase of glycine concentration were observed over time in the brain tissue near the injection site of the CGICs before solid tumors were detected by MRI. In contrast to the spectra of tumors grown from fresh cells, those from cultured cells showed intense peaks of lipids, increased absolute concentrations of glycine and choline-containing compounds, and decreased concentrations of glutamine, taurine and total creatine, when compared with a contralateral non tumor-bearing brain tissue. A decrease of concentrations of N-acetylaspartate and γ-aminobutyrate was found in both tumor phenotypes after solid tumor formation.
Conclusion: Our data show that this orthotopic mouse model of brain tumors seems to be suitable for studying glioma tumor as the changes in the metabolite concentrations at the injection site of the tumor cells are in an excellent agreement with metabolic changes observed in regions of tumor infiltration in patients (Stadlbauer A et al,. 2007). Further investigation are nevertheless needed to determine the cause of the dissimilarities between the tumors grown from cultured glioma-initiating cells and from freshly purified glioma-initiating cells, both derived from human glioblastomas. and precede the appearance of overt contrast on MR images.

PP 103

Clinical Assay Development Program

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Background: For molecularly guided cancer therapy to become reality, appropriately validated molecular assays are necessary. However, discoverers of predictive or prognostic molecular features often do not have resources to analytically validate their findings into a "locked down" assay.
Materials and Methods: The Cancer Diagnosis Program, NCI, has initiated a program composed of contracted CLIA accredited laboratories (Clinical Assay Development Network), a research laboratory (Molecular Characterization and Clinical Assay Development Center) at NCI-Frederick, and contracted Tissue Resources. Eligible applicants (industry, academia and government)need to have one justified defined intended clinical use, at least a prototype assay applicable to human tissues, and information on prevalence of the molecular feature in the disease to which the assay will be applied. Applicants describe the clinical need, the current state of the assay and future plans for assay development (such as use in clinical trial) and request CADP services for address analytical validation, transfer to quality environment, specimens for clinical validation, platform migration, etc. The applications, are evaluated by outside experts (Special Evaluation Panel − SEP). Those applications recommended by the SEP are reviewed internally to match resources and NCI strategic direction to the application. The successful application is then overseen by a project management team (project manager, subject matter expertise from NCI, expertise from contracted resources, and assay submitter). After validation, the standard operating procedures are returned to the assay submitter.
Results: Twelve applications were submitted at the first submission; one has been approved for resources from the first submission date. Project management is beginning. Common issues of the initial applicants were lack of definition of single intended use, or marker still in discovery. Clarification of the application instructions and discussions with the initial applicants were implemented.
Conclusion: Continued education of the marker development community is necessary to encourage development of potential molecular assays from the research lab into clinical use. The initial results of the program have been promising and interest in this program from potential applicants has been increasing (http://cadp.cancer.gov).

PP 66

Utility of VEGF and IL-6 as biomarkers for response to PTC299, a novel antiangiogenic

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Background: Solid tumor growth is dependent on angiogenesis, a process mediated primarily by vascular endothelial growth factor (VEGF). Current antiangiogenic agents sequester VEGF or block activation of VEGF receptors. On-target adverse events commonly observed with such treatments include hypertension, bleeding, and proteinuria. PTC299 is an investigational new drug that differs from existing therapies by inhibiting tumor production of VEGF and other angiogenic cytokines, including interleukin-6 (IL-6). PTC299 avoids inhibition of physiologic VEGF expression, potentially offering an advantageous safety profile relative to current anti-VEGF therapies.
Materials and Methods: A Phase 1b studying patients (pts) with advanced cancer evaluated PTC299 monotherapy Stages 1 and 2, or combination therapy with docetaxel Stage 3. A separate Phase 1b, studying pts with metastatic breast cancer evaluated PTC299 monotherapy Stage 1, or combination therapy with aromatase inhibitor Stage 2. In both studies, serum VEGF and IL-6 levels were assessed at baseline and end of Cycle 1(EOC1). Normal and elevated VEGF levels were defined as <300 and $\geqslant$300 pg/mL, respectively. Moderately elevated and highly elevated IL-6 levels were defined as <10 pg/mL and $\geqslant$10 pg/mL, respectively.
Results: The two studies enrolled 72 patients (15 males, 57 females) with median [range] age 60 [26−82] years, and ECOG PS 0 (n = 39) or 1 (n = 33). Paired VEGF and IL-6 baseline and EOC1 specimens were available from 58 and 48 patients. Patients with normal VEGF at Day 1 (n = 37) had a small mean (SD) increase in VEGF at EOC1 of 18 (122) pg/mL. Patients with elevated VEGF at Day 1 (n = 21) had a significant mean (SD) reduction in VEGF at EOC1 of −150 (188) pg/mL ($p = 0.001$). Patients with moderately elevated IL-6 at Day 1 (n = 25) had a mean (SD) increase in IL-6 at EOC1 of 6 (11) pg/mL. Patients with highly elevated IL-6 at Day 1 (n = 23) had a significant mean (SD) reduction in IL-6 at EOC1 of −13 (16) pg/mL ($p < 0.001$). No incidence of grade 3 or 4 hypertension, bleeding, or proteinuria occurred.
Conclusion: PTC299 offers a novel mechanistic approach to antiangiogenesis by selectively inhibiting pathological VEGF and IL-6 expression. This hypothesis is supported by the reduction of VEGF and IL-6 in patients with elevated levels at baseline and the adverse event profile observed to date.