and their cognate RNA (100, 10 ng) clustered together while controls and low RNA-samples (1, 0.5 ng) clustered separately. Gene expression variability was modest among replicates of samples with low RNA levels, but high among control samples. Gene Ontology analysis of genes exclusively expressed by captured spiked cells revealed an enrichment in those associated with ectodermic derivation and glandular function. AdnaWash treatment of controls reduces the expression of leukocytes genes. Comparison of genes expressed in spiked cells with those expressed in their cognate RNA revealed a consistent overlap (90%), whith samples derived from captured spiked cells (5% of all genes) enriched in genes associated to T-cells, B-cells and monocytes. Expression of key breast cancer genes (ER, EGFR, ERBB2) increased in spiked cells and their RNA compared to controls washed with standard or AdnaWash buffer while typically immune genes (CD3, CD8) were expressed at high levels only in samples not processed with the AdnaWash.
Conclusion: We have developed a protocol allowing to obtain reliable gene expression profiles from as low as 50 CTCs.

PP 13

Urine cell free DNA integrity as a marker for early diagnosis of non invasive bladder cancer

V. Casadio, C. Molinari, R. Gunelli, R. Silvestrini, M. Tebaldi, D. Amadori, D. Calistri. *Biosciences Laboratory, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola, Italy*

Background: Urine cell free DNA (UF DNA) has recently been proposed as a potential template for bladder cancer characterization and diagnosis. It is known that the origin of extracellular DNA can be established on the basis of its fragmentation; non cancer apoptotic cells produce highly fragmented DNA whereas necrotic cancer cells release longer DNA. The aim of our study was to verify the accuracy of a new non invasive approach in identifying bladder cancers. Attention was focused on three regions frequently amplified in bladder cancer corresponding to the genes c-MYC, BCAS1, HER 2. We also tested the integrity of cell free DNA in urine.
Materials and Methods: The study was conducted on a series of 132 individuals: 51 cancer patients, 46 symptomatic patients with benign urogenital diseases, and 32 healthy volunteers. After urine samples were collected, extracellular DNA was isolated from urine supernatant and free DNA integrity was determined blindly by three quantitative Real Time PCRs on three sequences longer than 250 bp: C-MYC, BCAS1 and HER2. A short fragment called STOX 1 was analyzed to exclude the presence of PCR inhibitors.
Results: UF DNA integrity analysis highlighted 0.1 ng/μl as the best cut-off value with 0.73 (95% CI 0.61–0.85) sensitivity, 0.84 specificity (95% CI 0.71–0.97) in healthy individuals, and 0.83 (95% CI 0.72–0.94) in symptomatic patients. The areas under the ROC curves were 0.8346 (95% CI 0.7391–0.9300) for healthy individuals and 0.7962 (95% CI 0.7070–0.8855) for symptomatic patients. In our case series UF DNA integrity showed higher sensitivity compared to cytology (0.73 versus 0.53) with the highest advantage for low-grade tumors (0.72 vs 0.15). The combination of cytology and UF-DNA analysis increased sensitivity to 0.81(95% CI 0.69–0.93).
Conclusion: Our preliminary data suggest that urine cell free DNA integrity has the potential to be a good marker for the diagnosis of early, non invasive bladder cancer. The diagnostic performance of the test did not vary significantly even when symptomatic individuals instead of healthy individuals were considered as reference group. Furthermore, the DNA analysis showed higher sensitivity with respect to cytology in detecting low-grade tumors, an essential element for early diagnosis. Research is ongoing in a larger case series to confirm these results.

PP 81

Alternative splicing studies for the identification of novel cancer markers

A-S. Casagrande, V. Kotraiah, D. Toema, D. Kong, F. Mahé, M. Pando, L. Desire. *Exonhit, Paris, France*

Background: Abnormal alternative splicing occurs in cancer, resulting in the production of novel transcript variants. Understanding the diverse mechanisms by which splicing dysregulation contributes to human disease will opened up new perspectives for drug development, biomarkers identification and drug response monitoring. ExonHit has generated a novel discovery platform, the Genome Wide SpliceArray™, and is currently building libraries of alternative splicing events that are deregulated in cancer and in cases of therapy resistance. These libraries can be interrogated for the identification of novel biomarkers, allowing to monitor disease status, progression/relapse, and specificity/selectivity of drug response. Here, the SpliceArray™ platform was used for the profiling of different cancers to identify novel markers that are either commonly regulated across multiple cancers or specific of a given cancer type.
Materials and Methods: Transcripts alternatively spliced were isolated from breast, colon, and lung tumors and their corresponding adjacent normal tissues. Different splicing patterns were evidenced in tumoral versus normal tissues and from specificity analysis performed across a pool of 20 normal organs. Based on combination of statistical analysis of probe sets deregulations, and protein knowledge, most relevant events were selected as alternatively spliced transcripts. Finally, focusing on splicing events that generate potential novel amino acid sequences, we conducted a QPCR expression analysis to validate the specificity of the selected events identified by the probe sets that emerged from the genome-wide splicing analysis in these 3 cancers.
Results: These validated events will be used to identify novel cell surface epitopes for antibody development with therapeutic and/or diagnostic usefulness. Applying this same approach, we profiled two types of Imatinib-resistant leukemia cell lines to identify pathways/genes or splicing events potentially involved in drug resistance affecting genes with biomarker potential.
Conclusion: Our results demonstrate that alternative RNA splicing offers a currently underexploited source of biological information for studying the cancer diversity. Platforms dedicated to alternative splicing can be integrated into discovery processes to allow identification of novel cancer makers, diagnostics and targets for drug discovery.

PP 14

mTOR inhibition decreases the malignant properties of cancer cells at selective stages of breast cancer progression in vitro

O. Cherednyk, A. Khoruzhenko, V. Kukharchuk, V. Filonenko. *Institute of Molecular Biology and Genetics, Kyiv, Ukraine*

Background: Kinase mTOR is one of the main links in signal transduction from variety of growth factors and hormones into the cell. mTOR participates in the regulation of protein synthesis, cell growth, proliferation etc. There are two functional complexes TORC1 and TORC2 whichregulate different cell events. Earlier it was demonstrated the overactivation of mTOR in numerous of malignant neoplasia. mTOR inhibitors are regarded as anti tumor drugs. But is not clear which stage of tumor progression is critically depended from mTOR activation/deactivation.
Materials and Methods: Immuunofluorescent analysis was applied to detect subcellular localization of mTOR in MCF-7 breast cancer cells (2D and 3D cultures) and postoperative specimens of human breast tumor. The effect of 1 and 10 nM of rapamycin on cultured cells was tested by MTT-test, adhesion and spreading assay, migration test using “wound healing” model, zymography, actin detection with falloidin, confocal microscopy.
Results: Immunoflurescent analysis find out predominantly cytoplasmic localization of mTOR in postoperative specimens of breast cancer and MCF-7 cells. Also, additional positive reaction for mTOR was evident in nucleoli. According to our information this mTOR positive staining of nucleoli is revealed for the first time. The process of tumor progression was hypothetically divided into several integral parts which were remodeled in vitro using breast cancer cell line MCF-7. Cell behavior under the condition of inhibited mTOR activity by rapamycin in concentration 1 and 10 nM was analyzed. It was detected the decrease of cell adhesion up to 40% at different time points. Besides, it was shown small but statistically significant reduction of cell spreading on the growth surface. In the condition of mTOR inhibition there was up to 80% decrease of cell migration in “wound healing” model. Therefore the effect of rapamycin on cell cytosceleton reorganization was determined. It was shown the apparent change in actin cytoskeleton organization in paranuclear space using falloidin detection of F-actin. In addition some decrease of MMP-9 activity in the presence of rapamycin was confirmed by zymography method.
Conclusion: There is the first evidence of mTOR presence in nucleoli. The most prominent effect of mTOR activity inhibition was observed in the assay of migratory potential of cancer cells, as well as on the cytoskeleton remodeling. Further study of the role of mTORα and novel splicing isoform mTORβ in tumor progression will be developed.

PP 33

Detection of prostate cancer by plasma proteome profiling based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

V. Chernyaev, V.E. Shevchenko, N.E. Arnotskaya, V.A. Chernyaev, V.B. Matveev. *Russian Cancer Research Centre, Moscow, Russian Federation*

Background: There is no satisfactory plasma biomarkers are available for the early detecting and monitoring of prostate cancer (PCa), one of the most frequent cancers worldwide. Serum prostate-specific antigen (PSA) levels have been widely used for diagnostic purposes but false-positive and false-negative results are still common. We hypothesize that PCa

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