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

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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

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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

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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

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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