an independent cohort of glioblastoma patients. We also compared the sensitivity and specificity of our novel real-time EGFRvIII detection assay to conventional RT-PCR and direct sequencing. We found that our assay can specifically detect EGFRvIII and can discriminate against wild-type EGFR in FFPE tumor samples. AQUA® analysis revealed that the presence of EGFRvIII transcript is associated with very high EGFR protein expression (98th percentile). Contrary to previous reports, only 44% of OSCC over-expressed EGFR in our study.
Conclusion: The EGFRvIII mutation is rare in OSCC. Our results corroborate previous reports of EGFRvIII expression only in tumors with extreme over-expression of EGFR. Our results suggest that EGFRvIII-specific therapies may not be ideally suited as first-line treatment in OSCC. However, EGFRvIII targeting might be a valuable addition to therapy in recurrent/metastatic OSCC where EGFRvIII may be over-represented due to the reduced responsiveness of EGFRvIII-positive tumors to conventional therapies. Since tumors expressing EGFRvIII are refractory to EGFR-targeted therapy, this could explain the poor success of EGFR targeting in clinical trials in recurrent/metastatic HNSCC patients. We conclude that highly specific and sensitive methods, such as the real-time RT-PCR assay and AQUA® analysis described here, are essential for the accurate assessment of EGFR mutation frequency and EGFR expression, and will facilitate the selection of optimal tailored therapies for OSCC patients.

PP 15

BRCA1 expression is required for efficacy of vinorelbine and is a predictive biomarker in malignant mesothelioma

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Background: Malignant mesothelioma is an aggressive tumor refractory to current therapies. Vinorelbine has been shown to exhibit useful clinical activity in mesothelioma. BRCA1 regulates sensitivity to microtubule poisons; however its involvement in regulating apoptosis in mesothelioma has not been investigated. The purpose of this study is to demonstrate that loss of BRCA1 confers resistance to vinorelbine induced apoptosis.
Materials and Methods: Dose-response curves were generated and BRCA1 expression was studied in a panel of 6 mesothelioma cell lines. Two resistant cell lines were also generated. The role of BRCA1 in regulating apoptotic response was shown by measurement of the percentage of apoptotic cell population and caspase 3/7 activity after transfection with siRNAs targeting BRCA1 or Caspase8. BRCA1 negativity percentage was also evaluated in 3 different cohorts of patients by immunostaining.
Results: Vinorelbine induced cytotoxicity correlated with BRCA1 expression level. The downregulation of BRCA1 expression by siRNA blocked caspase3 activation, PARP cleavage and the percentage of subG1 cell population. Moreover, when cells were selected for resistance to vinorelbine, this was associated with a reduction in BRCA1 expression compared to parental cells and re-expression of BRCA1 restored sensitivity. Data obtained after silencing of BAX and BAK showed that vinorelbine mediates toxicity irrespective of a functional mitochondrial apoptosis pathway; however the silencing of caspase8 decreased sensitivity. A high percentage of BRCA1 negativity was observed in primary mesotheliomas.
Conclusion: Our data highlight BRCA1 as a candidate predictive biomarker for vinorelbine induced apoptosis suggesting a potential utility in personalizing therapy with this agent.

PP 44

Myeloid zinc finger 1 regulates thymidylate synthase expression in patients with metastatic colorectal cancer showing the same promoter gene polymorphism

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Background: Background: Thymidylate Synthase (TS) is the target enzyme for fluoropyrimidine anticancer drugs. Its expression is regulated by the number of functional upstream stimulatory factor (USF) E box consensus elements present on its 5′ untranslated region. To date are known different polymorphisms, the first one consisting of 2 or 3 repeat of a 28 bp sequence, a further single nucleotide polymorphism (SNP) consisting in a G>C substitution within the second repeat of 3R (3RG>3RC) and recently it has been identified an additional SNP a G>C substitution at the 12th nucleotide in the first repeat of the 2R allele (2RG>2RC). These polymorphisms can influence TS expression, in particular 3R/3R genotype and the presence of 3RG alleles are associated to an increased transcriptional activity and to higher TS levels. The sequence of promoter region of colorectal cancer (CRC) samples was subjected to an in silico analysis (http://www.cbrc.jp/research/db/TFSEARCH.html) to search for all potential transcription factors binding this region. We found that Myeloid zinc finger 1 (MZF-1) binds the analyzed consensus. By the literature it is known that this factor induces invasion and in vivo metastasis in CRC, so we investigated a possible correlation between TS and MZF-1 expression in the same pathological samples.
Materials and Methods: Materials and Methods: we analyzed the distribution of these polimorphisms in a group of 68 healthy Caucasian subjects, and in the normal tissue, primary tumour and liver metastasis of 13 CRC patients. Tandem repeat length and the presence of SNP was determined by direct sequencing of genomic DNA. TS and MZF 1 expression were analyzed by immunohistochemistry.
Results: In healthy population the allele frequency was respectively 2RG(35%) 3RG (44%) 3RC (21%), in colorectal patients while both primary that normal and metastatic samples showed the same genotype: 2RG/3RG. TS and MZF-1 expression were related and gradually increased from normal tissue (negative) to the primary tumour (focally positive) in the metastases (overexpressing).
Conclusion: Conclusions: These unexpected results lead to the hypothesis of a genetic selection towards a more aggressive disease and enough suggest that regardless of genotype other factors are involved in regulation of TS expression as MZF 1, therefore the only genetic marker is not a valid predictor of eventual fluoropyrimidine response.

PP 77

Dissecting time- from tumor-related gene expression variability in the bilateral breast cancer model

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Background: Metachronous (MBC) or synchronous bilateral breast tumors (SBC) are generally distinct primaries, while pairs of primaries with local recurrences (LRC) share a common origin. Intra-pair biological variability in these three types of diseases results from combinations of time/host-related and genetic-related factors. Such clinical situation represents therefore an ideal model for trying to dissect tumor-related gene expression variability from time-related variability.
Materials and Methods: 18 pairs of synchronous, 11 of metachronous bilateral breast tumors and 10 pairs of primaries and locally recurrences were characterized with respect to gene expression profiles and similarity between pairs was measured using an intraclass correlation coefficient (ICC) computed for each gene. ICC distributions were compared for each type of tumor pairs using a Kruskal-Wallis test. No systemic treatment was administered between initial diagnosis and new disease manifestation in the subsets of women with MBCs or LRCs, whose primary tumors were all axillary node-negative.
Results: Considering all genes, the highest correlations were found for primaries and paired LRC and the lowest for MBC pairs. By limiting the analysis to the breast cancer intrinsic genes, correlations between primaries and paired LRC were enhanced while similar distribution were observed for SBC and MBC. On the opposite, using stromal-related genes there was a decrease of ICC values for MBC, which appeared significantly different from SBC.
Conclusion: Our data indicate that it is possible to dissect intra-pair gene expression variability into components associated with genetic origin or with time using specific gene subsets, in fact intrinsic genes are not influenced by the host and time, as instead happens for stromal genes.

PP 83

Gene expression profiling of circulating tumor cells in breast cancer

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Background: Enumeration of circulating tumor cells (CTC) in metastatic breast cancer predicts relapse-free survival and treatment failure while scanty data are currently available on their molecular features. Since CTCs might represent a surrogate tissue, their transcriptional characterization could likely allow to identify pathways involved in metastatic dissemination and to obtain clinically relevant information for monitoring prognosis and treatment response.
Materials and Methods: Predefined numbers (200, 100, 50) of MCF7 and MDA-MB-468 cells were spiked into blood from healthy donors captured using the AdnaTest EMT-1/Stem CellSelect kit (AdnaGen) and profiled (Illumina, DASL) in parallel with controls without cells and with RNA (100, 10, 1, 0.5 ng) from un-spiked cells. Controls were washed with PBS or with the AdnaWash buffer designed to improve leukocyte removal from captured cells.
Results: Gene expression detection rates for captured cells were around 60%. As expected detection rates dropped to lower values in control samples either washed with AdnaWash (30%) or with the standard washing buffer (45%). Samples derived from different numbers of spiked cells

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