

that define each neoplasia. The specificity of the assay is increased only if those DNA methylation markers that are always unmethylated in normal 'healthy' cells are included in this panel. In some cases, such as prostate cancer, a single hypermethylated marker, glutathione S-transferase-d (GSTP1), is informative in 80–90% of cases. For the various cancers for which DNA-methylation profiles are available, CpG-island hypermethylation has been used as a tool to detect cancer cells in all types of biological fluid and biopsy. One of its main advantages over other classical markers is the extreme sensitivity of some of the methods used for the detection of aberrant methylation. Another important finding has been that the CpG island hypermethylation of tumour-suppressor genes occurs early in tumorigenesis. This finding might be useful in early-detection screenings, especially in individuals with a high familial risk of developing cancer who have similar patterns of CpG-island hypermethylation as sporadic cases. Another interesting aspect are the use of DNA-methylation profiles as predictors of outcome. There are instances in which a tumour suppressor that undergoes methylation-associated silencing is a potential candidate for testing as a predictor of tumour prognosis. For example, death-associated protein kinase (DAPK), p16INK4a and epithelial membrane protein 3 (EMP3) hypermethylation have been linked to tumour prognosis in lung, colorectal and brain cancer patients. The final issue corresponds to pharmacoeugenetics: DNA methylation as a predictor of response to chemotherapy. The most compelling evidence that epigenomic profiles can predict responses of cancer to therapy is provided by the methylation-associated silencing of the DNA-repair protein MGMT in human brain tumours. MGMT is directly responsible for reversing the addition of alkyl groups to the guanine base of DNA and this base is the preferred point of attack in the DNA of several alkylating chemotherapeutic drugs, including BCNU (carmustine), ACNU (nimustine), procarbazine, streptozotocin and temozolamide. MGMT hypermethylation is the best independent predictor of response to BCNU and temozolamide in gliomas. The potential of MGMT to predict the chemoresponse of human tumours to alkylating agents can also be extended to other drugs with similar modes of action, such as cyclophosphamide.

Conclusions: DNA methylation biomarkers in human cancer are here to stay.

S18

Mining the proteome for clinically useful lung cancer signatures: Technology and trade-offs

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Introduction: Unlike some tumor types, the majority of the common solid tumors appear not to be driven by single dominant targetable pathways. Instead, diseases such as lung cancer are likely to be much more complex and heterogeneous, with many distinct and overlapping subsets of tumors within the class, each of which will demand an in depth analysis to define the optimal therapeutic approach. These groups are starting to be defined by multiple technologies, and the simplest example

is the small subset of patients with tumors expressing mutant EGFR, who achieve substantial clinical benefit from minimally toxic targeted therapy. Even for this small subset of patients with mutant epidermal growth factor receptors (EGFR), multiple resistance mechanisms have emerged requiring different salvage strategies. DNA sequence analysis will likely yield other small subgroups with direct therapeutic implications, and expression arrays are beginning to identify others, but analysis of the proteome has many theoretical advantages, for a complete knowledge of the proteome would encompass all known mechanisms of functional dysregulation associated with the development of cancer, including DNA mutations, rearrangements, transcriptional alterations and promoter methylation, but also post-translational modifications. This depth of information is still far from reality, however, and the true information content of today's technologies leaves a lot to be desired, but indications of utility are now being seen.

Main Message: Using the simple, inexpensive, and rapid technology of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI MS) we studied unfractionated, pretreatment sera to identify NSCLC patients with improved survival after treatment with the EGFR TKIs gefitinib and erlotinib. Mass spectra, independently acquired at two institutions, gave highly concordant results, and were used to generate an algorithm predictive of time to progression and survival. This prediction algorithm was then validated in a blinded manner in two independent cohorts of NSCLC patients treated with EGFR TKIs. This classification algorithm did not predict outcome in three independent cohorts of patients who did not receive treatment with EGFR TKIs. Thus, if upheld in prospective clinical trials, this analysis of pre-treatment peripheral blood might be useful in selecting therapy for advanced non-small cell lung cancer patients.

Conclusion: New technologies, such as shotgun proteomics, that give far more detailed information have also been far more cumbersome and less reproducible. However, we are now able to achieve a depth of information comparable to expression microarray analysis using shotgun proteomics of tumor and normal samples, with improving reproducibility. This is allowing for the more practical analysis of single samples, and definition of activated pathways in tumor cells in real-time. Direct quantitation of specific peptides of interest can also be achieved. It is likely that as the technology improves, proteomic signatures of cancer will be a significant source of information enabling the development of clinically useful individualized risk assessments and therapeutic decision-making.

S19

Diagnostic classification of pediatric cancers using microRNA profiles

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Introduction: While most conventional genes encode proteins to carry out their biological functions, the

recently discovered class of genes transcribing small non-coding RNAs, namely microRNAs, was found to play important regulatory roles in normal development and physiology in plants and animals. Mature microRNAs are 20–22 nucleotides molecules that can regulate gene expression through RNA interference effector complex (RISC) mediated mRNA degradation and translational suppression via complimentary pairing to predominantly 3'-untranslated region (3'-UTR) of their targeted messenger RNAs. Increasing number of studies has demonstrated a perturbation of the normal expression patterns of microRNAs in many human cancers.

Main Message: In order to investigate the microRNAs expression signatures in pediatric malignancies, we profiled a panel of pediatric tumor xenografts and cell lines belonging to 6 diagnostic categories using an in-house developed microRNA microarray. This panel included rhabdomyosarcoma (RMS), neuroblastoma (NB), Ewing's sarcoma, osteosarcoma, Wilm's and brain tumors. Each of our microRNA microarrays contains 336 human microRNAs probes based on Sanger Center's microRNA registry release 7. By unsupervised hierarchical clustering using all high quality data the cancers were primarily grouped according to their diagnosis. Using a parametric statistical analysis we identified 18 miRNAs that were differentially expressed between RMS and NB several of which were confirmed by real-time RT-PCR methods. We subsequently confirmed the expression of several cancer-specific microRNAs in the corresponding clinical tumor samples indicating their potential use for the diagnosis of these cancers.

In order to investigate the potential targets and functional roles of these microRNAs, we performed parallel gene expression profiling study on the same set of xenograft samples using Affymetrix U133 Plus 2 chips, and calculated the correlations between the expression of microRNAs and messenger RNAs. This resulting data matrix will identify potential targets of miRNA which will require experimental validation. Furthermore, we performed knock down and over expression studies with several NB and RMS specific miRNAs. We found that the over expression of mir-133a caused a profound growth suppression and induction of apoptosis in a RMS cell line.

Conclusions: Our findings showed the potential use of microRNAs for the diagnosis of pediatric cancers. Furthermore our results will reveal new insights into the function, and identify potential therapeutic uses of these small RNAs in these pediatric cancers.

S20

Novel biomarkers for prognosis and therapy response in ovarian cancer

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Introduction: New biomarkers for diagnosis, prognosis, monitoring and prediction of therapeutic response in ovarian cancer are needed.

Main Message: Recently, we have delineated the complete organization of the human kallikrein locus, which

comprises 15 genes with significant homology. We have produced recombinant proteins and developed ELISA methodologies for quantitative assessment of all kallikrein proteins in serum and tissue extracts. We found that some tissue kallikreins, including KLK5, KLK6, KLK8, KLK10, KLK11 and KLK13 are over-expressed in ovarian cancer tissue, in comparison to normal ovarian tissue. We found that these KLKs have prognostic value in ovarian cancer. More recently, we have analyzed panels of kallikreins, as well as other biomarkers, in our efforts to develop a multiparametric prognostic and predictive model for this disease. We developed a combined marker which provided an area under the curve (AUC) of 0.97 for discriminating normal from cancer groups. Univariately, KLK5 and KLK6 were positively associated with progression. Increasing levels of KLK13 were associated with chemotherapy response with an odds ratio of 2.32. The predictive power of KLK13 to chemotherapy response was improved with a panel of 5 biomarkers with AUC of 0.75. After adding other clinical parameters, the AUC increased to 0.91. This data suggests that a group of kallikreins and multiparametric combinations with other biomarkers and clinical parameters can significantly assist with ovarian cancer classification, prognosis and response to chemotherapy.

Conclusions: In conclusion, human tissue kallikreins represent novel biomarkers for ovarian cancer diagnosis, prognosis and prediction of response to chemotherapy. These biomarkers, assessed individually and/or in combination with other biomarkers, may assist with delivery of individualized treatments in ovarian cancer.

S21

Biomarkers of brain tumors to temozolamide treatment

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Introduction: Glioblastomas are the most malignant brain tumors with a median survival of less than 15 months despite modern therapies. The accumulation of diverse aberrations in regulatory processes enables tumor cells to bypass the effects of most classical therapies available. Molecular alterations underlying such mechanisms comprise aberrations on the genetic level, such as point mutations of distinct genes, or amplifications and deletions, while others result from epigenetic modifications such as aberrant methylation of CpG islands in the regulatory sequence of genes.

Main Message: In a candidate gene approach the epigenetic inactivation of the MGMT gene was evaluated as a predictive factor for benefit from the alkylating agent temozolamide that was added concomitant and adjuvant to radiotherapy. MGMT is a repair enzyme known to rapidly revert the highly toxic O6-methylguanine to its native state, guanine, in a suicide reaction – hence, blunting most of the treatment effect of the alkylating drug. The translational