Speaker Summaries

SP140

Gene methylation biomarkers for lung cancer risk, recurrence, and prognosis

S. Belinsky. *Lovelace Respiratory Research Institute, USA*

The involvement of gene methylation in carcinogenesis has led to studies focused on establishing the utility of methylation as a biomarker in screening for cancer risk, prevention, treatment, and prognosis. Our group was the first to establish that gene-specific methylation could be detected in sputum from lung cancer patients prior to clinical diagnosis of lung cancer. This observation led to the initiation of studies focused on identifying a panel of genes whose methylation in sputum would predict the presence of early lung cancer. Those studies conducted through the Johns Hopkins Lung SPORE and in collaboration with the Colorado Lung SPORE initially identified a panel of six genes whose concomitant methylation in sputum was associated with a 6.5-fold increased risk for lung cancer. Studies have now been extended to identify additional genes associated with lung cancer risk, to assess whether methylation can predict tumor recurrence, and survival.
The nested, methylation-specific PCR (MSP) assay was used to detect gene promoter methylation in sputum, while standard MSP assayed for methylation in primary tumors.
An additional 46 genes have been evaluated for their association to lung cancer using the case-control design within the Colorado Cohort. We have now identified 14 genes associated with a 2-fold or more increased lung cancer risk. These studies were extended to evaluate the performance of the top 17 genes in a study of prevalent Stage I cases and controls. Similar associations of genes to lung cancer were observed. The ability of gene methylation to predict tumor recurrence was also assessed in sputum from resected Stage I lung cancer patients participating in a Phase III trial of selenium supplementation. Findings indicate both individual and composite methylation of a gene panel were associated with recurrence. Finally, the methylation status of SULF2, a heparin sulfate 6-O-endosulfatase enzyme that promotes the release of growth and angiogenic factors, was defined in lung tumors. Survival following tumor resection and in patients undergoing chemotherapy was reduced in tumors with an unmethylated SULF2 gene. These studies highlight the promise of gene promoter methylation as a biomarker for early detection, recurrence, and prognosis for lung cancer patients.
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SP139

Leukemic stem cells

D. Bonnet. *Cancer Research UK, London Research Institute, UK*

An emerging concept in cancer biology is that a rare population of cancer stem cells exists among the heterogeneous cell mass that constitutes the tumour. Based on this notion, tumours are thought to be driven by a cellular subpopulation that retains key stem cell features. Yet, despite their critical importance, much remains to be learned about the developmental origin of cancer stem cells and the mechanisms responsible for their emergence in the course of the disease.
The adaptation of xenotransplantation assays to examine the propagation of AML in vivo has allowed the phenotypic identification of the AML-IC. Transplantation of primary AML cells into NOD/SCID mice led to the finding that only rare cells, termed AML-initiating cells (AML-IC), are capable of initiating and sustaining growth of the leukemic clone in vivo, and serial transplantation experiments showed that AML-IC possess high self-renewal capacity, and thus can be considered to be the leukemic stem cells.
The development of an in vivo model that replicates many aspects of human AML had provide a mean to identify leukaemic stem cells. This in vivo assay provides the foundation of an assay to define the biological and molecular properties of such leukaemic stem cells (LSC).
Since the early studies, further heterogeneity in the LSCs has been identified. Using cell-tracking analysis, the Dick's group identified different sub-clone of SL-ICs. Recently, we also show phenotypic heterogeneity of the SL-ICs between patients and also within the same patients. This heterogeneity not only indicates a potential differential origin or progression of the disease but also have important implications in the development of new therapies to eradicate these cells.
Existing cancer therapies have been developed largely against the bulk population. The lack of durable response in most cases, suggests that the treatment used may not effectively target the CSC population. Indeed, the failure of the current therapeutic regimens is likely related to the resistance and persistence of CSC. Thus, the identification of CSC has important implications for future research as well as for the development of novel therapies.
The seminar will summarise our knowledge of Cancer Stem Cells notably in AML and will try to propose few new avenues that might be taken to eradicate these population of cells.

SP149

Validation of statistically reliable biomarkers

M. Buyse. *IDDI, Belgium*

Background: The clinical literature is replete with the use of biomarkers, but in many cases these have not been properly validated, resulting in a large number of false claims, inappropriate trial designs, and sub-optimal patient management. Yet biomarkers hold great potential for the development of new and more effective cancer treatments by targeting patients who benefit and by providing early signals of efficacy.
Definitions: A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". The term biomarker covers characteristics measured at baseline as well as those measured repeatedly over time, before, during or after treatment. Clinical data, laboratory data, imaging data, gene expression and proteomic data can all be considered biomarkers.
Types of biomarkers: Biomarkers can be useful as prognostic factors that predict the outcome of individual patients in terms of a clinical endpoint, predictive factors that predict the effect of a specific treatment on a clinical endpoint in groups of patients, or surrogate endpoints that replace a clinical endpoint of interest. Biomarkers can be used to stratify the patients at entry in clinical trials, to select the patients eligible for clinical trials, to monitor patients and guide treatment decisions, or to substitute for a clinical endpoint in the evaluation of the effects of new treatments.
Study designs: Different study designs are required for the identification and validation of biomarkers. Case-control or cohort studies are sufficient to validate prognostic biomarkers, large randomized trials are needed to validate predictive biomarkers, and multiple randomized trials are needed to validate surrogate biomarkers. In all cases, the biomarker should be validated either through cross-validation (internal validation in the discovery set) or in different trials (external validation in a confirmatory set).
Validation criteria: The criteria used to validate biomarkers include classification measures (sensitivity and specificity, ROC curves), treatment effect measures (odds ratios, hazard ratios) association measures (correlation coefficients, information theory based measures), and prediction measures (the surrogate threshold effect). These various criteria all have advantages and limitations that will be illustrated in this presentation.

SP141

Serum proteomics in lung cancer

D. Carbone. *Vanderbilt University, USA*

Unlike some tumor types, the majority of the common solid tumors appear not to be driven by single dominant targetable pathways. DNA sequence analysis will likely yield 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.
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. We are currently in the process of testing this signature in sample sets from past randomized clinical trials, and a prospective trial is underway. New technologies, such as shotgun proteomics, we are now able to achieve a depth of information comparable to expression microarray analysis, 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 in the serum as candidate biomarkers 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 of risk assessments and therapeutic decision-making.

SP158
The use of autoantibodies in the early detection of cancer

C. Chapman. *The University of Nottingham, UK*

Measurement of cancer associated antigens (eg CA15-3, CA125, CEA) in serum are often performed but, as they are essentially markers of disease bulk, they are of limited use in the early identification of a cancer. Early detection, at a stage when the tumour is still localised and treatable, is the goal of any screening tool and new approaches are required which do not rely on already circulating tumour cells, or disease bulk.
Cancer cells often present a number of novel, aberrantly expressed or mutated proteins, or even abnormally large amounts of normal proteins. The immune system is uniquely adapted to detect such changes and even small quantities of such proteins can lead to the production of a specific immune response in the form of specific autoantibodies. A very small tumour bulk, that could not be measured using conventional tumour marker assays, could therefore be identified following measurement of such antibodies.
Due to the heterogeneous nature of most solid tumours the measurement of autoantibodies to only one cancer associated antigen is unlikely to be sufficiently sensitive to make this approach useful as a screening test. Whereas measurement of autoantibodies to a panel of such antigens, if correctly managed, could provide a simple tool that is both sensitive and specific.
Autoantibodies to cancer antigens have been shown to be detectable in a number of different solid tumours. In some cases these autoantibodies have also been identified 4−5 years before the cancer could be diagnosed using more routine methodologies (eg mammography for breast cancer and CT for lung cancer).
Recent work has reported that approximately 40% of lung cancers can be detected by measuring autoantibodies to a range of tumour associated antigens, when compared to an age, gender and smoking matched group of 'normal' individuals (with a 90% specificity). This panel identified both small cell (SCLC) and non-small cell lung cancers (NSCLCs) and also picked up both early and late stage disease. Work is ongoing to try to identify a different panel of antigens which will be useful in the earlier detection of other solid tumours like breast, colorectal and hepatocellular carcinomas.
It will also be interesting to determine whether measurement of such antibodies following surgical resection and treatment, may also provide prognostic information for the clinician.

SP153
Stem cells and breast cancer: treatment resistance, markers and novel therapeutic targets

R. Clarke. *Paterson Institute, University of Manchester, UK*

There is emerging evidence that breast cancer stem cells (CSCs) are resistant to radio, chemo and endocrine therapies suggesting that CSC-specific treatments are needed. We investigated breast CSCs and established that breast cancer cell lines and primary tumours contain a CSC population that can be enriched for using cell surface markers such as ESA+CD44+CD24low.
Due to their relative insensitivity to treatment, we and others have demonstrated that CSCs are also enriched by radio, chemo and endocrine therapy. Increases in the proportion of CSCs after therapy is measured using the above markers and mammosphere colony assays of stem cell activity. DNA repair, survival and stem cell signalling pathways are strong emerging candidates for the underlying mechanisms of resistance.
With regards to endocrine treatment, we have established that CSCs in oestrogen receptor-β-positive (ER+) breast cancer are ER− and therefore inherently resistant to the direct effects of endocrine therapies. However, CSCs still respond to therapy-induced changes in microenvironmental signals. One candidate pathway known to regulate normal stem cells is Notch receptor signalling.
The Notch pathway comprises five secreted ligands, Jagged1/2 and Delta-like 1/3/4 and four receptors, Notch1−4. In breast cancer, we have shown that this pathway is activated by oestrogen and inhibited by tamoxifen and faslodex. We therefore investigated Notch receptor signalling within the CSC population and tested the effects of Notch inhibition on stem cell activity in breast cancer.
We have evidence that activated Notch4 is higher than activated Notch1 in CSCs, compared to the differentiated populations. Notch inhibition using gamma secretase inhibitors (GSI) had no significant effect on the cleavage of the Notch4 receptor but potently inhibited signalling through Notch1 receptor. GSIs caused decreased CSC activity in vitro, and reduced the growth of MCF7 and MDA-MB231 tumours by up to 50%. However, blocking all four Notch receptors using Numb cDNA or specific knockdown of Notch4 using shRNA completely prevented breast tumour formation.
Our findings indicate that Notch4 plays a key role in tumour initiation by CSCs while Notch1 is more active in differentiated proliferation. Thus, therapies targeting Notch4 receptor are likely to be more effective in preventing treatment resistance than those targeting Notch1.

SP165
Optimizing information obtained from fine needle aspiration (FNA) biopsies

D. Clark. *Johns Hopkins Medical Institutions, USA*

Fine Needle Aspiration Biopsy (FNAB) is a minimally-invasive and cost-effective method for sampling human tumors that is widely used around the world. Historically, FNAB samples have provided adequate material for microscopic examination; however, the successful development and application of molecularly targeted agents (MTAs) against cancer will also demand the robust and reliable detection of novel molecular biomarkers in FNAB samples. Molecular characterization of FNAB samples has been relatively limited and typically confined to a single molecular marker analyzed in a fixed sample. Expansion of such studies to more comprehensive analyses, such as gene expression profiling or multiplexed protein arrays, would significantly enhance cancer research and clinical diagnostics. However, such studies will require preservation of biospecimen "information content" through specialized specimen handling as well as sensitive, multiplexed analytical platforms.
FNA samples offer several advantages over surgically-excised or core biopsy samples: 1) Obtains viable cells; 2) Allows immediate assessment of specimen for adequacy; 3) Minimal preanalytical variability; 4) Can be performed repeatedly over time, permitting temporal studies within a single animal or human; 5) Less invasive and more cost-effective than surgical excisional biopsies. Challenges to the molecular analysis of FNAs include the small number of cells and the heterogeneity of the cellular composition. Potential technological solutions to these challenges will be presented.
One additional opportunity presented by the FNA sample is functional profiling of live cells through ex vivo biomarkers. The term "ex vivo biomarker" has been used to define a novel class of biomarkers − those which are evoked by live tumor cells after they have been removed from the patient. This involves removing viable cells from a patient through an FNA then stimulating the cells in vitro with growth factors that are relevant to the signal transduction networks targeted by MTAs. The biomarkers are typically newly modified phosphoproteins or newly expressed mRNAs in the signaling network. Such assays offer exciting possible applications: 1) patient stratification based on functional information to inform clinical trial design or clinical management; 2) novel pharmacodynamic assays for use in the development of targeted therapies.

SP175
Application of high resolution mass spectrometry for cancer biomarker discovery and validation

T. Conrads. *University of Pittsburgh, USA*

Developments in high resolution mass spectrometry (MS) and nanoflow chromatography have made possible high-throughput proteomic investigations of myriad clinically relevant samples in the expectation of identifying peptide or protein biomarkers for disease. Conventional protein biomarker discovery investigations are predominantly performed with samples such as serum or plasma. While serum or plasma samples may be more desirable from a clinical standpoint, tissue likely possesses a greater abundance of readily identifiable proteins directly reflective of disease. This lecture