Proffered Paper sessions

Basic Science/Translational research
Thursday 24 September 2009, 09:00–11:15

11LBA LATE BREAKING ABSTRACT

Cell reprogramming by hypoxia

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Background: Low oxygen tension (hypoxia) is an important determinant in tumor progression. In this study we assess the prognostic value of an in vitro derived hypoxia gene signature in neuroblastoma patients.
Material and Methods: L1-L2 regularization framework has been applied on gene expression profiles of 11 neuroblastoma cell lines to define the neuroblastoma hypoxia signature. We applied k-means clustering on the expression level of the signature 62 probesets to segregate 88 neuroblastoma patients and subgroups obtained by common risk factors stratification. We analyzed the classes by Kaplan–Meier curves and log-rank test for overall survival (OS) and event-free survival (EFS). Multivariate Cox analysis was performed to define the predictive power of the signature.
Results: The neuroblastoma hypoxia signature distinguished two groups of neuroblastoma patients classifying them as poor prognosis (21 patients), those having OS rate of 25.5% and EFS rate of 27.7%, and as good prognosis (67 patients), those having OS rate of 73.2% and EFS rate of 67.7%. The poor prognosis patients show an over-expression of the hypoxia probesets. Multivariate Cox analysis revealed that the neuroblastoma hypoxia signature is a significant independent predictor after controlling for commonly used risk factors. When applied to MYCN not amplified patients, the hypoxia signature was capable to stratify patients with OS rate of 24.2% and EFS rate of 27.3% for the patients with poor prognosis, compared with OS rate of 81.4% and EFS rate of 74.8% for the patients with good prognosis.
Conclusions: We demonstrate that the NB-hypo signature is a significant prognostic factor capable of stratify neuroblastoma patients. Furthermore, we obtained the proof of principle that the approach of hypoxia genes selection from in vitro controlled tumor cell lines, is a feasible method to identify specific contribution of the microenvironment to the tumors' biology.

Gastro-intestinal malignancies – Colorectal I
Monday 21 September 2009, 11:00–13:15

12LBA LATE BREAKING ABSTRACT

A Plasma-based colorectal cancer (CRC) screening assay using DNA methylation markers – first results of multicenter studies

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Introduction: We have developed a plasma-based DNA methylation test for CRC screening. This report summarizes the marker selection, sample preparation, analytical validation and first results within prospective multicenter studies.
Material and Methods: *Marker identification and selection*: Using epigenetic re-expression profiles of colon cancer cell lines, candidate genes were identified and subsequently screened using tissue samples from CRC and controls.
Clinical studies: In the *CRC.SCR.1* study, 2200 persons were recruited in a CRC screening setting for collection of pre-colonoscopy plasma samples and associated clinical data. In the "*Molecular Signatures in Colorectal Carcinoma* (MSKK)" study pre-surgery plasma samples were collected from patients with pathologically proven diagnosis of CRC. Both studies, sponsored by Signature Diagnostics in collaboration with OncoMethylome Sciences, were/are executed in accordance with Good Clinical Practice (GCP) guidelines including approval by ethics committees; all persons gave written informed consent prior to sample collection. The CRC.SCR.1 samples were complemented with MSKK samples in a fashion that guaranteed a stage distribution expected in a CRC screening setting.
Methylation assays: Isolation of cell free plasma DNA and bisulphite modification was done on 3.3 ml of EDTA plasma from a single blood draw with the QIAamp Circulating NA and Epitect kits (Qiagen). Real time Methylation Specific PCR (MSP) on ABI7900 cyclers used Molecular Beacons as the detector probes.
Results: DNA extraction and bisulfite conversion methods resulted in recovery of about 40% of the input DNA. Two newly reported methylation markers, SYNE1 and FOXE1, each gave more than 85% sensitivity in tissue samples of all CRC stages while showing excellent specificity in lymphocytic DNA. A duplex MSP assay was designed processing half of the extracted plasma DNA. The first results for 242 controls and 22 cases showed 94% specificity and 68% sensitivity for SYNE1 only and 91% specificity and 77% sensitivity for the combination of SYNE1 and FOXE1.
Conclusions: A CRC diagnostic assay, with high specificity and sensitivity, using plasma has been developed which is well designed for commercial product development. Based on the obtained results, the CRC.SCR.1 study has been extended with the aim of enrolling a second cohort of 5500 persons by the end of 2009.

13LBA LATE BREAKING ABSTRACT

S100A4 transcripts in blood of colon, rectal, and gastric cancer patients: development of a new blood-based assay for improved diagnosis and prognosis

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Background: This prospective clinical study was conducted to define the diagnostic and prognostic power of S100A4 transcripts detected in plasma samples from colon, rectal, and gastric cancer patients. S100A4 is known to be a metastasis progressor, and detection of S100A4 mRNA in several primary tumors is of diagnostic and prognostic relevance. A blood based-assay for detection of S100A4 transcripts is not available so far.
Material and Methods: Altogether, 466 blood samples of tumor patients were examined, with 185, 190, and 91 samples of colon, rectal, and gastric cancer patients, respectively. Blood was collected daily from hospitalized patients and from the outpatient care. Blood samples of 51 tumor-free volunteers, collected in two independent cohorts, served as controls. Following isolation of RNA, S100A4 mRNA was determined by quantitative gene-specific two-step real-time RT-PCR.
Results: S100A4 mRNA was detected in all plasma samples analyzed at significantly higher levels in colon, rectal, and gastric cancer patients compared to the tumor-free volunteers ($P < 0.0001$, respectively). Patients of each entity were sub-classified due to disease stages. Every sub-cohort demonstrated significantly higher S100A4 transcript plasma levels than healthy volunteers. Furthermore, significantly elevated S100A4 levels were detected in plasma of metastasized colon cancer patients compared to non-metastasized patients. Interestingly, prospectively analyzed follow-up patients of all entities who later developed metastases showed clearly higher S100A4 levels at the initial blood taking compared to the non-metastasized follow-up patients.
Conclusion: Here we provide for the first time a reliable and simple blood-based assay for transcript quantification of the metastasis-promoting gene S100A4 in patients' plasma. We demonstrate its applicability for identification of occult tumors and/or metastasis in seemingly healthy populations, and in newly diagnosed or already treated patients. Moreover, the prognostic value of S100A4 transcript for metastasis formation in follow-up patients is shown.