D6-02 Molecular Biology & Prognostic Factors, Thu, 12:30 - 14:15

Patient-to-patient and tumor sub-sample variations of gene expression in NSCLC

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Background: Human lung tumors are known to be considerably heterogeneous in respect to histology across the tumor. The purpose of this study was to compare the patient-to patient and intra-patient tumor sub-sampling variations in gene expression profiles.

Methods: In this prospective study tumor samples from 20 patients who underwent resection for primary lung cancer were collected (4 squamous cell carcinomas, 11 adeno carcinomas and 5 mixed type adeno-squamous carcinomas). They were 16 males and 4 females with a median age of 63 years (range 38-69). The pathological stages were found to be IA in 4 cases, IB in 10 cases, IIA in 1 case and IIB in 5 cases. 9 tumors were grade II and 11 tumors grade III. Tissue samples were snap frozen in liquid nitrogen within 20 minutes after resection and stored at -80°C until analysis. Serial cryo-sections were prepared from tissue samples of 4 different sites of the tumor for each patient. The first and the last section of a series were HE stained and analyzed for tumor cell composition. Only samples with more than 50% viable tumor cells were used for RNA extraction and subsequent expression profiling. All RNAs were analysed with Agilent 2100 Bioanalyzer and RNA 6000 nano assay kit. The median RIN number was 8.9. Microarray analyses were carried out using Affymetrix Human Genome U133 Plus 2.0 arrays according to Affymetrix standard protocols. All measurements have undergone an extensive QC on probe level (BG signal, detection and masking of signal artefacts, etc.) and probe set level (model fit quality, affinity profiles, RNA degradation analysis, etc.). A Roche in-house developed design of the array has been taken to eliminate wrong and non-specific probes. The probe sets have been re-designed and re-annotated according to the latest builds of human genome and transcriptome. RMA style probe level model (PLM) has been used to fit probe-intensity data and estimate probe set expression.

Results: Inter-patient (patient-to-patient) and intra-patient (tumor site to tumor site) variations of gene expression have been determined in a uni-variate setting using linear mixed effect model approach. The probe sets showing consistently larger intra-patient differences as compared with the variation due to workflow and that of patient-specific expression have been identified. Considering the probes sets (genes) which have found to be responsible for significant sampling variations, gene ontology analysis has shown that these genes are 'at random' and are functionally irrelevant. No association to TNM status, tumor stage, patient gender/age, or other study co-variates has been detected. Several genes have been found to be associated with stromal tissue. As also confirmed by multi-variate analysis (PCA, PLS), 98% of our probe sets showed significant patient-to-patient, but no sampling variation.

Conclusions: Gene expression profiles based on single tumor tissue blocks are representative for the whole tumor, if viable tumor cell content is equal to or greater 50%.

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Proliferation and 'invasiveness' gene-expression signatures predict survival of surgically treated non-small-cell lung cancer

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Introduction: Non-small cell lung cancer (NSCLC) is a leading cause of cancer mortality in both men and women. One way to improve the outcome is to predict survival at diagnosis, in order to select patients for the most appropriate treatment. As microarray data have already shown to be able to predict the outcome, we hypothesized that further refining the prognostic value of these profiles would be possible by selecting them according to biological characteristics such as proliferation and hypoxia.

Methods: Several in vitro derived published and unpublished gene-expression based signatures were tested on a clinical patient microarray dataset. This dataset consists of 86 surgically treated NSCLC patients of which complete follow-up data was available.

The previously published wound signature, invasiveness gene signature (IGS) and Chi signature (hypoxia) as well as an unpublished signature for proliferation were evaluated with a signature score. This score was defined as the weighted average expression of the genes in the signature. This score is used to evaluate the predictive accuracy of the various signatures based on Kaplan-Meier survival analysis, log-rank tests and multivariate Cox-regression analysis.

Results: Only two of the evaluated signatures, IGS and proliferation signature, had significant predictive value in Kaplan-Meier survival analyses. Stratifying patients in groups based on the score of these signatures resulted in a clear difference in survival, p-values of log-rank test were 0.039 and 0.018 for the IGS and proliferation signature respectively. The areas under the curves for 2-years survival were 0.64 for the IGS and 0.66 for the proliferation signature. Further multivariate Cox-regression analysis with backward stepwise selection procedure showed that the proliferation signature was the only signature that was an independent predictive parameter ($p = 0.030$).

Conclusions: The proliferation signature is a predictor for survival of surgically treated patients with NSCLC in univariate and multivariate analysis, while the IGS was only significant in univariate analysis. This information may further enable the selection of patients for adjuvant therapy as well as give further insight to the mechanisms underlying the bad prognosis.