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POSTER

Gene Expression Profiling of Lung Adenocarcinoma Stage I Patients: Risk for Relapse Disease

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Background: Lung cancer is the leading cause of cancer-related death in the world wide. Nearly 50% of patients with stages I and II non-small cell lung cancer (NSCLC) will die from recurrent disease despite surgical resection. No reliable clinical or molecular predictors are currently available for identifying those at high risk for developing recurrent disease. As a consequence, it is not possible to select those high-risk patients for more aggressive therapies and assign less aggressive treatments to patients at low risk for recurrence. The aim of the study was to identify novel genes involved in the risk of early relapse (ER) compared to no relapse (NR) disease from lung adenocarcinoma stage I patients.

Material and Methods: From tissue banking of 110 consecutive resected NSCLC patients at S.M. della Misericordia Hospital in Perugia-Italy, we only selected frozen specimens of lung adenocarcinoma tissue from stage I patients.

We compared gene expression profiling from Normal Lung (NL) and cancer specimens from NR and ER, using Affimetrix human microarray HG-U133Plus 2.0. We applied principal component analysis (PCA) combine with clustering methods to select the significant genes. We validated selected genes up-regulated and down-regulated by quantitative-PCR (Q-PCR).

Results: Microarray analysis had shown a panel of 223 differentially expressed genes (84 up- and 139 down-regulated). Based on the fold change ratio of ER vs NR, we selected 51 genes (20 up- and 31 down-regulated). The results of genes expression in Q-PCR were superimposable respect to those of microarray analysis ($p=0.0038$). The 51 selected genes were evaluated one by one in the 18 patient samples (13 NR and 5 ER) by Q-PCR: 74.2% and 80% of the up- and down-regulated genes, respectively, were predictive for clustering patients in ER and NR.

Conclusion: Our results indicate that it is possible to define, through gene expression, a characteristic gene profiling of early relapse tumour patients with an increased risk of relapse disease. Among the identified genes (up-regulated: INSL4, CLCA2, FABP3, GLYATL2, IL1RL1 and down-regulated: XIST, OLFM4, GSTA1, SCGB1A1, IGHG) several are already known in tumour pathways and others could be new potential targets. To further validate our results we will use an independent cohort of patients with lung adenocarcinoma stage I and the analyses are ongoing and they will be presented at the conference.

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Plasma Metabolomics in Non-small-cell Lung Cancer

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Background: The aim of this study was to determine whether the plasma metabolomic profile would be able to discriminate patients with non-small-cell lung cancer (NSCLC) vs cases with active benign diseases, and within the NSCLC group if changes in the plasma metabolome would be correlated with any clinical characteristic, including histology, stage, smoking status, survival.

Materials and Methods: Sample population consisted of 300 subjects including patients with stage I-IV NSCLC (62%) and control cases with benign lung diseases (38%). The two groups were matched by gender and age.

In the NSCLC group blood samples were obtained prior to any anticancer treatment. Plasma metabolome was profiled using gas chromatography coupled to mass spectrometry (GC-MS). Bioinformatics was based on multivariate Orthogonal-Partial Least Square analysis, and proposed models were internally validated by seven-fold cross validation.

Results: Based on the relative abundance of 218 metabolites we were able to discriminate NSCLC cases vs controls (predictive coefficient $Q^2=0.1$; $p<0.001$) and stage IV NSCLC vs stage I-II disease ($Q^2=0.22$; $p<0.001$). Similar models (i.e. early vs advanced stage) could also be built separately in patients with either non-squamous tumours ($n=93$; $Q^2=0.25$; $p<0.001$)

as well as in squamous-cell carcinomas ($n=29$; $Q^2=0.37$; $p=0.003$). Interestingly, the variables mainly responsible for these latter two models consisted of different metabolites (correlation coefficient R^2 between the two models = 0.39), separately and specifically linked to the two histology groups.

Finally, plasma metabolome was not modulated by smoking status (never/former smokers [55%] vs current smokers [45%]) in neither patient group (all cases, NSCLC, controls).

Conclusion: These preliminary findings indicate that squamous and non-squamous tumours are modeled separately suggesting different effects on the plasma metabolome. The results in general are promising and warrant further data analysis. The identification of metabolites is ongoing.

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Dicer and Drosha Expression and Outcomes in Patients With Lung Cancer

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Background: We have studied Dicer and Drosha, components of the RNA-interference machinery, in lung cancer tissues. It is possible that deregulated miRNA expression, observed in lung tumours is secondary to defective RNA silencing machinery.

Material and Methods: A total of 115 non-small cell lung cancer tumour tissues were collected. Samples were harvested from patients who have been treated primarily by surgical resection at Hospital 12 de Octubre (Madrid, Spain). Histologic preparations of tumour sections were examined by pathologists without any information about the outcome. The histologic groups included are: lung adenocarcinoma (AC) ($n=46$), squamous (SCC) ($n=56$), large cells (LC) ($n=8$) and others ($n=7$). All tumour specimens were collected under approval from the institutional review boards and ethics committees of Hospital 12 de Octubre, with written informed consent from each patient. Total RNA was extracted from tumour samples (TRI Reagen, Ambion), followed by purification (RNeasy Mini-Kit, Qiagen). TaqMan quantitative real-time PCR was done according to the manufacturer's instructions (7500 Applied Biosystems). Relative gene expression values were calculated by the Second Derivative Maximum Method 4.0 software. To determine the distribution of Dicer and Drosha levels around cutoff points, histograms were created on the expression ratio. Kaplan-Meier plots were constructed and a log-rank test was used to determine differences among survival curves according to Dicer and Drosha expression level.

Results: Levels of mRNA varied among cancer specimens. We used the median to divide into two subpopulations the distributions of Dicer and Drosha mRNA levels: 4.08 as the cutoff value for high and low Dicer, and 1.61 as the cutoff value for Drosha. In 29.6% of specimens, there were decreased levels of both Dicer and Drosha mRNA. Specimen with increased mRNA levels had a median ratio for Dicer of 5.39 (range, 4.09 to 12.65) and a median ratio for Drosha of 2.12 (range, 1.65 to 10.81). Low Drosha mRNA levels were significantly associated with increased overall survival ($p=0.012$) and increased progression free survival ($p=0.036$).

Conclusions: Drosha mRNA expression is significantly associated with survival, indicating that its level in lung cancer tumours could be clinically relevant.

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Identification of KRas Mutational Polymorphism by Molecular Analysis Using High-resolution Melting System in a Large MCRC Mexican Sample

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Background: Mutations in K-Ras oncogene are frequently found in human cancers and particularly in colorectal cancer. These mutations can indicate prognosis and maybe predictive of drug response. It has been demonstrated that the successful treatment of metastatic Colorectal Cancer (mCRC), using monoclonal antibody therapy is directly linked to the oncogenic activation of the K-ras signaling pathway. In order to