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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 agenes (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, IGHD) 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 $Q2 = 0.1$; $p < 0.001$) and stage IV NSCLC vs stage I-II disease ($Q2 = 0.22$; $p < 0.001$). Similar models (i.e. early vs advanced stage) could also be built separately in patients with either non-squamous tumours ($n = 93$; $Q2 = 0.25$; $p < 0.001$) as well as in squamous-cell carcinomas ($n = 29$; $Q2 = 0.37$; $p = 0.003$). Interestingly, the variables mainly responsible for these latter two models consisted of different metabolites (correlation coefficient R2 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 seaparately 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

determine the mutational status and polymorphism frequency of KRAS gene in Mexico, we analyzed a large sample of mCRC.
Material and Methods: We retrospectively collected paraffin embedded tumours from 807 patients with mCRC diagnosis from different hospitals in Mexico. Mutation detection in KRAS codons 12 and 13 were determined. The tests were performed in a LightCycler® 2.0 system using the LightMix® Kit KRAS mutation (TIB-Molbiol, Germany).
Results: Four hundred ninety-five patients were KRAS wild-type tumours (61%) and 312 patients were KRAS mutated (39%); mutated tumours shown the following polymorphism: Gly12Ser (GGT>AGT) (47%; n = 148); Gly12Asp (GGT>GAT) (16%, n = 50), Gly12Val (GGT>GTT) (7%; n = 22); Gly13Asp (6%, n = 20), Gly13Cys (5%, n = 16), Gly12Arg (4%, n = 13), Gly12Cys (4%, n = 12), Gly12Ala (4%, n = 11).
Conclusions: KRAS mutation status frequency (39%) was not out of range by previous reports in others countries or regions (35−55%). However, the most common mutation polymorphism was Gly12Ser (GGT>AGT) (47%), in contrast with previous reports that indicated Gly12Asp (GGT>GAT) as the most common polymorphism. The mutational polymorphism associated to higher recurrence risk and mortality by RASCAL II study was Gly12Val (GGT>GTT); surprisingly, in our study it was of low frequency mutation type (7%). Outcome measures in KRAS wild-type patients treated with a cetuximab-based regimen at the moment are not evaluated because of the early treatment stage.

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High-sensitive D-dimer Determination for the Prediction of Chemotherapy-associated Venous Thromboembolism in Lung Cancer Patients

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Background: Venous thromboembolism (VTE) is an important cause of morbidity and mortality in lung cancer patients, especially during the first 3−6 months of chemotherapy. Several studies have been undertaken to identify novel candidate biomarkers to improve risk prediction, namely D-dimer (DD). However, despite its determination has become a cornerstone in the exclusion of clinically suspected VTE, its predictive use in cancer yielded controversial results. The recent availability of a high sensitive (HS) assay for DD determination prompted us to investigate whether baseline HS-DD determination could be useful to predict VTE risk in lung cancer patients scheduled for chemotherapy (adjuvant or first-line).
Material and Methods: 108 consenting patients (65±9 years) with newly diagnosed (22) or metastatic (86) lung (11 SCLC, 97 NSCLC) cancer were enrolled. Exclusion criteria were: ECOG score >2, prophylactic or therapeutic doses of any heparin, use of anticoagulant drugs. HemosIL DD and DD HS 500 (Instrumentation Laboratory) immunoassays were performed on an ACL TOP coagulometer on citrated plasma samples obtained before chemotherapy.
Results In a median 6-months follow-up, VTE occurred in 16 (15%) of 108 patients with a median time-to-event of 1.8 months. Higher median DD (780 vs. 411 ng/ml, $p = 0.002$) and HS-DD (3507 vs. 1055 ng/ml, $p = 0.0002$) levels were found in patients who developed VTE. Despite a correlation between DD and HS-DD levels ($r = 0.837$, $p < 0.0001$), a significant proportion of negative DD patients scored positive for HS-DD (18%, $p < 0.0001$) using conventional cutoffs (280 and 500 ng/ml, respectively). ROC curves generated from continuously distributed test results showed a better area under the curve for HS-DD (0.816, SE = 0.05) than DD (0.762, SE = 0.07). Based on these results, a cutoff value of 1500 ng/ml was calculated for HS-DD resulting in a sensitivity 0.81, specificity 0.69, NPV 0.96 (OR for VTE: 9.4, $p < 0.0001$). Multivariate analysis showed that HS-DD ($p < 0.0001$), but not DD, was an independent predictor for VTE. Finally, Cox proportional hazard survival analysis demonstrated a RR of 10.1 ($p < 0.0001$) of developing VTE during chemotherapy for patients with baseline HS-DD levels >1500 ng/ml.
Conclusions: These results demonstrate that the use of an enhanced immunoassay for D-dimer determination prior to chemotherapy is able to predict VTE in lung cancer out-patients with a substantial gain in accuracy over conventional testing.

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Overexpression of MiR-141 and MiR-126 Distinguishes Metastatic Castration Resistant Prostate Cancer (mCRPC) From Localized Prostate Cancer (PCa) and Controls in Human Plasma

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Objective: MicroRNAs (miRNAs) are small, non-coding RNA molecules implicated in the pathogenesis of several malignancies. Several miRNAs exhibit dysregulated expression in PCa, but little is known about the clinical utility of this. We sought to establish a miRNA signature in PCa by comparing levels of expression of miRNAs in the plasma of men with varying stages of PCa.
Methods: Plasma samples were prospectively collected from 75 subjects (25 controls, 25 localized PCa, 25 mCRPC). Candidate miRNAs were chosen based on increased expression in the mCRPC group compared with the local and control groups from a miRNA panel qPCR analysis on the pooled samples of each group. Expression of 8 candidate miRNAs was validated using qPCR performed on individual subject samples.
Results: From the pooled analysis 8 candidate miRNAs were chosen: miR-141, miR-375, miR-200a, miR-9, miR-126, miR-152, miR-200c, and miR-21. qPCR performed on individual samples revealed that overexpression of miR-141, miR-375, and miR-126 was seen in mCRPC and that the combination of miR-141/miR-126 overexpression was able to consistently distinguish between mCRPC vs. local or controls. Among mCRPC samples marked overexpression of miR-126 was observed in those patients who had not been treated with docetaxel. Of the 3 patients who were treated with abiraterone acetate (AA) as part of an open label clinical trial, all had clinical responses and all showed marked overexpression of miR-141 and miR-126.
Conclusions: In this exploratory study, candidate miRNAs that have been associated with prostate cancer pogression were detectable in human plasma that distinguished between mCRPC vs. local/control samples. miR-126, associated with angiogensis and inflammation, was upregulated in patients with progressive mCRPC who had not previously been exposed to docetaxel. Overexpression of miR-141, which has been associated with androgen receptor upregulation, may be a potential predictive biomarker for next-generation antiandrogen therapy. Further studies are planned.

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Sphingosine Kinase 1 Correlates With a Neuroendocrine Phenotype in Breast Cancer in Vivo and in Vitro

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Background: Neuroendocrine differentiated (NE) breast tumours are diagnosed by their diffuse expression of the NE markers synaptophysin (SYP), chromogranin A (CgA) and neuron-specific enolase (NSE). However the genesis of differentiation to this phenotype in the breast has not been uncovered. Sphingosine kinase 1 (SK1) is a lipid kinase whose bioactive product, sphingosine-1-phosphate has been associated with tumour growth and proliferation, negative prognosis and refraction to endocrine therapy in breast cancer. Recently it was suggested that SK1 is involved in the NE differentiation of LnCAP prostate cells. We have investigated the correlation of SK1 expression with NE differentiation.
Materials and Methods: MCF7 breast tumour cells were stably transfected with SK1 and pellets were formalin fixed and paraffin embedded. 50 formalin fixed NE breast tumour samples were collected from patients admitted for surgery to San Giovanni Battista and San Luigi Hospitals of Torino (Italy). SK1 and the NE markers SYP, CgA and NSE were detected using immunohistochemistry and immunofluorescence.
Results: MCF7 cells do not express detectable levels of the NE markers, SYP or NSE. Over-expression of SK1 in MCF7 cells resulted in an induction of expression of both SYP and NSE. Investigation of NE breast tumours revealed that they display a unique, diffuse SK1 expression pattern when compared to other breast tumours and that SK1 colocalises with CgA.
Conclusions: Results from this case study indicate that SK1 is a potential marker of NE differentiation in breast cancer. SK1 over-expression is sufficient to induce SYP and NSE expression in MCF7 breast tumour cells, suggesting that SK1 has a causative role in NE differentiation.