Detection of viral induced double-stranded RNA intermediates in archival paraffin blocks

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Double strand RNA (dsRNA) species were previously thought to be a ‘junk DNA’ equivalent are related to endogenous retroviral elements (ERV) inserted into the human genome. Genomic integration sites and blocking of these processes have been well documented in HIV and Hepatitis C related diseases. Viral integration into human genome has been shown in a range of solid tumors, but mechanisms less understood. The detection of dsRNA in the research setting has been largely limited to cell line models and controlled transfections. To better characterize function significance of ERV and interactions at the cellular level, we outline efforts to detect and quantify such viral genomic elements with a focus on archival clinical samples in the commonly stored paraffin block. Tissue blocks issues with clinical and biochemical documented viral infection and associated viral cytopathic changes were selected from the Department of Pathology and Biorepository and standard 4 micron sections were prepared. Two (2) commercially available antibodies raised to dsRNA fragments (J2 and K1 antibodies) were directly compared by chromogenic antibodies raised to dsRNA fragments (J2 and K1 antibodies) were directly compared by chromogenic and immunofluorescent methods. In addition, downstream biomarkers as identified by literature searches include RIG-1 and IRF 7 as moderate probable success and MDA-5 and STAT 1 as higher level success. Correlative analysis of downstream pathway markers with labeling results of dsRNA antibodies suffers from lack of sensitive measures of dsRNA fragments as opposed to the downstream cellular pathway markers. This lack of concordance likely reflects the short nature of dsRNAs in decade old paraffin tissue and/or differences in abundance. Future work will focus on developing more sensitive probes or IF probe signal amplification.

Conclusions: Mexican NSCLC patients exhibit a specific mutation profile with alterations in several cancer-related genes mainly in EGFR, TP53, GNAQ, HNF1A, VHL and KRAS. The NGS technology showed greater sensitivity than PCR to detect relevant mutations in NSCLC. Several mutations found in high incidence in this study could become relevant targets for novel targeted therapies in NSCLC.

Prediction of distant recurrence-free survival in resectable lung adenocarcinoma

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Background: Recurrence and lung cancer mortality in resected, early stage lung cancer is likely due to the presence of micro-metastatic disease not detected during surgery or the pre-surgical assessment. The likelihood of such latent disease increases with higher stages and thus treatment with adjuvant therapy is currently determined by pathological stage. However, significant recurrent disease and cancer-related mortality even in stage I patients suggest that pathological stage alone does not adequately identify resected patients that could benefit from further treatment. Tumor molecular features can improve stratification of patients for increased risk of distant recurrence.

Methods: Formalin-fixed surgical samples from 318 stage I and II lung adenocarcinoma patients, treated with surgery alone, were examined for the expression of 31 proliferation genes (CCP score) by multiplex, quantitative RT-PCR. A prognostic score (PS) was calculated from pathological stage and the CCP score. The added prognostic value of the CCP score for the prediction of five-year distant recurrence-free survival and five-year cancer-specific survival was tested in Cox proportional hazards regression models with adjustment for clinical parameters. Estimates of disease-free survival and cancer-specific survival were calculated by the Kaplan-Meier method.

Results: Among 289 samples with successful expression analysis, 85 had developed a distant recurrence at five years post-surgery. After adjustment for age, gender, tumor size and pleural invasion, pathological stage (HR 2.28, 95%CI 1.16-4.50 for IA vs IB, p=0.0015) and the CCP score (HR 1.62 per IQR, 95% CI 1.15-2.29, p=0.0055) were independent predictors of distant recurrence and as well as for cancer-specific survival [pathological stage (HR 1.72, 95%CI 0.93-3.17 for IA vs IB, p=0.024), CCP score (HR 1.40 per IQR, 95%CI 1.03-1.90, p=0.031)]. The prognostic score was a better predictor of distant recurrence than pathological stage alone (PS: HR 2.22 95%CI 1.11-4.44, p=0.023, stage: HR 1.70 95%CI 0.86-3.55