harbored Kinesin Family Member 5B ALK (KIF5B-ALK) fusion, 4 samples harbored huntingtin interacting protein 1 ALK (HIP1-ALK) fusion, 1 sample harbored kinesin light chain 1 ALK (KLC1-ALK) fusion, and the remaining 16 samples harbored novel fusion variants. Of the 133 EML4-ALK positive samples, 55 samples harbored E13:A20 variants (Variant 1), 55 samples harbored E6:A20 (Variant 3), 16 samples harbored E20:A20 (Variant 2), 4 samples harbored E18:A20 and 3 samples harbored E2:A20.

Conclusion: This is the largest molecular epidemiological study to describe ALK fusion variants in East Asian LUAC patients. EML4 is the most common fusion partner of ALK gene. About 84% of ALK fusion positive patients harbor EML4-ALK, and 5.7% harbor non-EML4-ALK fusions. The remaining 10% harbor fusions with unknown partners. For EML4-ALK, E13:A20 and E6:A20 are the most common variants, comprising more than 80% of EML4-ALK fusion. E20:A20, E18:A20 and E2:A20 are also identified in East Asian patients. The constitution of EML4-ALK fusion variants is similar to the previous reports of patient cohorts in western countries. All the ALK fusion variants mentioned should be included in the LUAC fusion screening assays.

Mutation status matters: RAS, p53, and targeting autophagy as a potential strategy in NSCLC

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The hyperglycolytic, hypermetabolic activity of cancer cells contributes to a 'stressful' local tumor environment in which cells compete for limited nutrients while being bathed in excessive metabolic waste, including high levels of lactic acid. Cells within a tumor undergo a period of environmental stress that is most pronounced when the growing tumor cell mass exceeds its vasculature, further limiting nutrients and increasing the accumulation of waste. This can be viewed as a selection step in which only those cells, both cancer and stroma, that are best adapted to survive the stress maintain the ability to proliferate. The aim of this study was to characterize the molecular basis for the survival of non-small cell lung cancer (NSCLC) cells when exposed to environmental stress. To accomplish this objective we used an in vitro culture system designed to recapitulate the environmental stressed conditions of depleted nutrients and accumulated waste experienced by cells within tumors. In examining a panel of human NSCLC cell lines, we unexpectedly found that the status of RAS was a more important determinant for surviving environmental stress than the status of p53. Lines with activating RAS mutations (KRAS or NRAS) were better poised to survive severe environmental stress, independent of p53 status. In detailed molecular cell biological studies comparing a NSCLC cell line wild type for p53 with an activating KRAS mutation to a NSCLC cell line mutant for p53 without an activating RAS mutation, we found that survival correlated with the level of autophagy in the unstressed cells. Cells that survived stress had low basal levels of autophagy that increased upon stress, whereas those that did not survive stress had elevated autophagy in the absence of stress that was increased further with the stress. Basal activation of AKT (also known as Protein kinase B) was higher in unstressed KRAS cells than in p53 mutant cells. Active AKT inhibits autophagy, thereby likely explaining the lower basal level of autophagy in KRAS mutant cells. In quantitative fluorescence microscopy studies of living cells, we demonstrated that p53 mutant NSCLC cells, in addition to having elevated autophagy, have more acidic and motile lysosomes, phenotypes correlated with increased lysosomal activity. This basal 'lysosomal activation' is likely linked to the elevation of basal autophagy. Based on these studies we conclude that the ability of cells to maintain low autophagy in the absence of stress and to activate autophagy in response to stress are important for enhanced survival when cells are challenged with environmental stress. Activated RAS, via regulation of nutrient absorption pathways, might make cells less dependent on stress-activated mechanisms, such as autophagy, to fulfill nutrient needs. Furthermore, loss of p53 in the absence of KRAS activation results in an increased basal autophagy, which when further increased by environmental stress, results in decreased cell survival. These data suggest that the role of autophagy in cancer cell survival in the context of environmental stress is linked to specific oncogenic mutations. This finding has significant implications for targeting autophagy in the treatment of lung cancer.

Unique roles for Akt isoforms in lung cancer

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Lung cancer is the leading cause of cancer-related mortalities worldwide and 5 year survival rates are typically $<$20%. As traditional therapies are largely ineffective

against advanced lung cancer, molecular targets are being explored. An emerging molecular target for lung cancer is the signaling molecule Akt as it is frequently activated in lung cancer. Akt is activated by a number of growth factor receptors and mediates processes such as proliferation, survival, migration, and metabolism. Three Akt isoforms (Akt1-3) exist in mammals and it remains unclear whether each isoform has distinct functions. To evaluate the function of Akt isoforms in lung cancer, a transgenic mouse model (SPC-IGFIR) where lung tumors were induced by elevated expression of IGF-IR and subsequent activation of Akt was used. SPC-IGFIR mice were mated with *Akt1*$^{-/-}$ or *Akt2*$^{-/-}$ mice to produce SPC-IGFIR mice lacking either Akt1 or Akt2. Lung tumorigenesis was suppressed in SPC-IGFIR/*Akt1*$^{-/-}$ mice and enhanced in SPC-IGFIR/*Akt2*$^{-/-}$ mice. Lung tumor cells in SPC-IGFIR/*Akt2*$^{-/-}$ mice appeared to infilitrate the lungs to a greater extend than either SPC-IGFIR or SPC-IGFIR/*Akt1*$^{-/-}$ tumor cells which had a more nodular appearance. RNA sequencing revealed a number of genes and transcripts differentially expressed in the SPC-IGFIR/*Akt2*$^{-/-}$ lung tumors and several of these genes have been implicated in human NSCLC. Using 2 human NCSLC cell lines it was determined that an AKT1 selective inhibitor impaired cell survival more than an AKT2 inhibitor or a pan-AKT inhibitor. These results suggest that inhibition of Akt1 may represent a therapeutic strategy for lung cancer.

Whole blood FPR1 mRNA expression identifies both non-small cell and small cell lung cancer

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Purpose: Although long-term survival rates for early stage lung cancer are high, most patients are diagnosed with advanced stage disease. The National Lung Screening Trial demonstrated a survival benefit with annual low-dose chest CT (LDCT) in patients at high risk, despite a false positive rate of 96%. Most attempts at development of blood-based early detection for lung cancer employ panels of several biomarkers, which are susceptible to overfitting and poor reproducibility on new samples. We hypothesized that a single biomarker-based approach would be more effective.

Methods: Whole blood was collected in PAXgene tubes from 289 patients under IRB-approved protocols at four institutions. This included 231 retrospective specimens and 58 specimens collected prospectively. We collected smoking history, cancer diagnosis, age and gender for all patients. Due to the use of a single marker and low risk of overfitting, the training set consisted of only 29 patients. The validation set consisted of 133 non-small cell lung cancer, 14 small cell lung cancer and 113 cancer-free patients. A total of 11 patient cohorts were created based on combination of cancer histology and smoking history. RNA was extracted, and the expression of formyl peptide receptor 1 (FPR1) and a reference gene were quantified by an automated one-step Taqman RT-PCR assay.

Results: In the validation set, elevated levels of FPR1 mRNA in whole blood demonstrated a sensitivity of 55% and a specificity of 87% for lung cancer detection. Among prospectively collected specimens, FPR1 mRNA had a sensitivity of 68% and specificity of 89%. The sensitivity of prospective samples was significantly higher than retrospective samples ($p=0.018$) while the specificity was unchanged. We observed that longer times between collection and refrigeration/freezing of samples tended to yield negative results. This time was much shorter on prospective samples, and this likely explains the increased sensitivity. No significant difference in sensitivity was observed based on histology (including small cell vs. non-small cell) or cancer stage. Results from patients with benign nodules were similar to healthy volunteers. We used multiple data mining techniques and found no meaningful relationship between our test results and any clinical characteristic other than lung cancer diagnosis, including age, smoking history, and gender.

Discussion: FPR1 mRNA levels in whole blood can identify the presence of lung cancer with high accuracy. A single-marker test is less prone to overfitting of data when compared to a multi-marker test created through complex data mining algorithms, and thus is more likely to be reproducible. The use of a training/validation set strategy further increases likelihood that results will be reproduced in follow-on studies. Using FPR1 as a reflex test for suspicious lung cancer screening CT scans may have the potential to increase the positive predictive value (PPV). Future planned studies will explore the source of this biomarker and evaluate its effectiveness in augmenting CT scans for patients undergoing lung cancer screening.