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Evaluation of the Mutant KRAS-Driven NSCLC Tumor Immune Microenvironment Using Patient-Derived Cell Line Xenografts in a Humanized Mouse Preclinical Model for Development of New Immunotherapy Approaches
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Purpose of the Study: Tumor cell and tumor microenvironment (TME) features that influence the response to immune checkpoint blockade in lung cancer are incompletely defined. We wanted to develop preclinical models of lung cancer to explore the relationship of non-small cell lung cancer (NSCLC) molecular characteristics (oncogenotype such as mutant KRAS and LKB1/STK11, and mRNA expression profiles) to the human TME. Experimental Procedures: We exploited the use of 8 (all KRAS mutant) molecularly characterized (whole-exome seq mutation and RNAseq analyses) patient-derived NSCLC lines grown as xenografts in vivo in humanized NSG-SGM3 mice in an effort to determine the effect of different NSCLCs on the immune landscape of lung cancer xenografts. The triple transgenic NSG-SGM3 (NSGS) mice express human IL-3, GM-CSF, and SCF, which combine the features of the highly immunodefficient NOD scid gamma (NSG) mouse with cytokines that support the stable engraftment of human myeloid lineages and regulatory T-cell populations. Subcutaneous KRAS mutant-driven non-NSCLC xenografts grown in NSG-SGM3 mice “humanized” with CD34+ cord blood cells were subjected to immune landscape analysis through flow cytometry, multiplex immunohistochemistry, and cytokine analysis. The xenografts were also treated with various combinations of checkpoint inhibitors, radiation, and activation of the innate immune pathway with emricasan (pan caspase inhibitor).

Summary of New Data: Our results show that the 8 KRAS mutant NSCLCs each were associated with a different spectrum of human TME; 3 of the 8 xenografts had only 0.1% of immune cell infiltrates while other xenografts had 20% immune cell infiltrates; that tumor mutation burden (TMB, absolute mutation calls 233 to 2,076) is not predictive of CD8+ T-cell infiltration in KRAS mutant-driven NSCLC xenografts. In NSCLC xenografts with high CD8+ T-cell infiltrate, the CD8+ cells in the TME were not activated, resulting in limited responses to PD-1/PD-L1 therapy. However, stimulation of the cGAS-STING innate immune pathway with emricasan followed by radiation (15 cGy) resulted in dramatic anti-tumor response. Conclusions: NSCLC xenografts grown in “humanized” mice show great intertumor heterogeneity effects on the TME even within the KRAS mutant subgroup, and it is possible to demonstrate targeted therapy such as emricasan/radiation can lead to changes in improved antitumor responses.

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Identification of Th1 Epitopes in Lung Non-Small Cell Lung Cancer Antigens to Develop a Multiantigen Vaccine
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Non-small cell lung cancer (NSCLC) represents 85% of all lung cancer cases and it is highly smoking related. The goal of this project is to develop a vaccine for lung cancer prevention in current or past smokers by identifying immunogenic proteins in lung cancer that are able to induce a potent inflammatory Th1 response. Lung cancer has one of the highest mutation rates of all types of cancer, but driver mutations that could be targeted for a vaccine for lung cancer prevention are unknown. Gene expression profiling of bronchial biopsy specimens from smokers has shown that changes in gene expression in histologically normal epithelia can discern people with and without lung cancer. Many of these changes are proteins aberrantly upregulated, but not mutated. We have used quantitative mass-spectroscopy analysis to identify proteins overexpressed in NSCLC cell lines compared with normal lung epithelial cells. Five NSCLC cell lines (three squamous cell carcinoma and two adenocarcinoma) and two normal lung epithelial cell lines were included in the analysis. A total of 14,219 peptides, corresponding to 2,875 proteins, were identified. We selected for further analysis those proteins identified with >95% confidence and at least 3 peptides per protein, and overexpressed in three or more NSCLC cell lines. We considered that a protein is overexpressed if [expression in the NSCLC cell line/ expression in the normal cell line] >1.5. A total of 154 antigens met our criteria. Candidate antigens were investigated by siRNA screening to identify those genes with a function in maintaining cell tumor growth.