Some cancer prognoses have been radically improved in recent years, but little headway has been made with others. One of these diseases, small-cell lung cancer (SCLC), has a five-year survival rate of less than 7% and a standard of care that has been essentially unchanged for forty years. One promising avenue to improve SCLC outcomes is to understand the cancer’s underlying genetic alterations that drive its formation and growth. Functional inactivation of the Rb gene is seen in a number of cancers and is a genetic hallmark of SCLC. Normally Rb promotes differentiation by regulating lineage-specific transcription factors, including pluripotency factors such as Sox2. However, there is evidence that when certain tissues lose Rb, Sox2 becomes upregulated and promotes oncogenesis. To understand this relationship in the pursuit to uncover new treatments for SCLC, we have studied the role of Sox2 in Rb loss-initiated tumors by investigating both the tumor initiation in a SCLC genetically engineered mouse model, as well as tumor maintenance in SCLC cell lines and organoid culture.

**B26**

Relationship of Sox2 and Rb in Tumor Initiation and Maintenance in Small-Cell Lung Cancer

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Some cancer prognoses have been radically improved in recent years, but little headway has been made with others. One of these diseases, small-cell lung cancer (SCLC), has a five-year survival rate of less than 7% and a standard of care that has been essentially unchanged for forty years. One promising avenue to improve SCLC outcomes is to understand the cancer’s underlying genetic alterations that drive its formation and growth. Functional inactivation of the Rb gene is seen in a number of cancers and is a genetic hallmark of SCLC. Normally Rb promotes differentiation by regulating lineage-specific transcription factors, including pluripotency factors such as Sox2. However, there is evidence that when certain tissues lose Rb, Sox2 becomes upregulated and promotes oncogenesis. To understand this relationship in the pursuit to uncover new treatments for SCLC, we have studied the role of Sox2 in Rb loss-initiated tumors by investigating both the tumor initiation in a SCLC genetically engineered mouse model, as well as tumor maintenance in SCLC cell lines and organoid culture.

**B27**

IHH Acts as a Tumor Suppressor of Lung Adenocarcinoma by Repressing Reactive Oxygen Species

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**Background:** Aberrant activation of the Hedgehog (Hh) signaling pathway, a crucial developmental pathway, drives the tumor growth of basal cell carcinoma, medulloblastoma, and rhabdomyoma. However, recent data suggest that paracrine activation of the pathway is tumor suppressive rather than oncogenic in sporadic epithelial cancers. The role of the pathway in non-small lung cancer is poorly understood. Thus, we explored the role of stromal Hh pathway activation in growth of lung tumor epithelia.

**Methods:** Human and murine lung adenocarcinoma cell lines and murine fibroblasts were used to probe SHH mRNA and protein expression and to verify paracrine activation of the Hh signaling pathway. The role of paracrine SHH was tested in vivo using KrasLSL-G12D/;Trp53fl/fl (KP) and LSL-KrasG12D/;Trp53fl/fl, Shhfl/fl (KPS) autochthonous murine lung cancer models. The role of IHH was examined in vivo using the pSCECRISPR system in KP;Rosa26SL-IHH/+. Tumor growth monitored by bioluminescence imaging. Results: In human lung adenocarcinoma (LAD) patients, higher expression of SHH mRNA correlated with poor overall and progression free survival. Coculture of high SHH-expressing tumor epithelial cells and Shh-Light2 reporter fibroblasts demonstrated that SHH activated the Hh pathway in the fibroblasts in a paracrine manner. Surprisingly, genetic loss of SHH in an autochthonous mouse model, KPS, did not affect overall survival compared to KP mice. However, early inhibition of stromal Hh pathway by 5E1, an anti-SHH/IHH antibody, in KP mice resulted in significantly worse survival with increased metastatic burden. We tested the loss of IHH in vivo with the pSCECRISPR system. IHH-loss in airway epithelia led to more aggressive tumor growth, suggesting that IHH, not SHH, activates the pathway in stroma to drive its tumor suppressive effects—a novel role for IHH in the lung. Tumors from mice treated with 5E1 had decreased blood vessel density and increased reactive oxygen species (ROS). Treatment of KP mice with 5E1 and N-acetylcysteine, as a ROS scavenger, decreased tumor ROS levels, inhibited tumor growth, and prolonged mouse survival, suggesting that increased ROS levels from stromal Hh pathway inhibition accelerated lung tumor growth. **Conclusions:** IHH activates the Hh signaling pathway in lung stroma in a paracrine manner to suppress tumor growth and metastases, in part, by limiting ROS production.

**B28**

Intermittent Hypoxia Exacerbates Tumor Progression in a Mouse Model of Lung Cancer


**Background:** Obstructive sleep apnea (OSA) is a very prevalent disorder characterized by chronic intermittent hypoxia (CHI), and some reports suggested that OSA is related to increased incidence of cancer as well as cancer progression. The purpose of this study was to evaluate whether obstructive sleep apnea (OSA)-related chronic intermittent hypoxia (CHI) influences lung cancer progression and to elucidate the associated mechanisms in a mouse model of lung cancer. Methods: C57/B6 mice in a CHI group were exposed to intermittent hypoxia for two weeks after tumor induction and compared with control mice (room air). Hypoxia inducible factor 1α (HIF-1α), vascular endothelial growth factor (VEGF), and metastasis-related matrix metalloproteinases (MMP) were measured. The expression levels of several hypoxia-related pathway proteins including HIF-1α, Wnt/β-catenin, the nuclear factor erythroid 2-related factor 2 (Nrf2), and mammalian target of rapamycin-ERK were measured by Western blot. Results: The number (P < 0.01) and volume (P < 0.05) of tumors were increased in the CHI group. The activity of MMP-2 was enhanced after CHI treatment. The level of VEGF was increased significantly in the CHI group (p < 0.05), β-catenin and Nrf2 were translocated to the nucleus and the levels of downstream effectors of Wnt/β-catenin signaling increased after H exposure. Conclusions: CHI enhanced proliferative and migratory properties of tumors in a mouse model of lung cancer. β-Catenin and Nrf2 appeared to be crucial mediators of tumor growth. These results suggest evidence for the causal link between OSA and lung cancer progression.

**B30**

The Role of SMARCA4 as an EGFR-Independent Mechanism of Resistance to Osimertinib


Targeted therapies have replaced conventional chemotherapy as first-line treatment for patients with nonsmall cell lung cancers harboring epidermal growth factor receptor (EGFR) alterations. Although tyrosine-kinase inhibitors (TKI) targeting these proteins lead to responses in ~70% of cases, tumors almost inevitably become resistant. Acquired resistance is commonly caused by secondary mutations in the target oncogene, activation of bypass signaling pathways, histologic transformation of the tumor, or unknown mechanisms (~20-40%). Epigenetic mechanisms are responsible for regulating genes involved in cell lineage specificity, and they are known to modulate tumorigenesis. In recent years, several epigenetic modifiers have also been implicated in processes related to drug resistance. We hypothesized that dysfunction of epigenetic processes plays a role in mediating resistance to TKIs. To examine this possibility, we generated three isogenic osimertinib-sensitive/resistant cell line pairs and mined whole-exome and RNA sequencing (RNA-seq) data. Distinct alterations and phenotypes were identified in the different models, highlighting the importance of the baseline biologic context for the type of osimertinib resistance
B31
Development of Multicell-Type Organoid Cultures for Preclinical Studies of Immunotherapeutics for Lung Cancer

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Introduction/Purpose of Study: Macrophages are key regulators of the immune landscape within the tumor microenvironment (TME). The plasticity of macrophage phenotypes in the TME has previously been correlated with prognosis within non-small cell lung cancer (NSCLC). Depending on their phenotype, macrophages in the TME can secrete protumour cytokine and chemokines, ultimately suppressing the function of other immune cells in the TME. The purpose of our study was to explore the ability of individual NSCLC preclinical models to alter macrophage phenotype in organoid cultures and to relate effects on macrophages to the molecular characteristics of different NSCLCs. We hypothesize that immune suppression occurs through tumor-secreted signaling molecules, and if blocked, macrophage suppression can be alleviated, resulting in a better antitumor immune response. Experimental Procedures: We developed an in vitro organoid coculture system (NSCLC tumor cells, human cancer-associated fibroblasts, CAFs, and mouse macrophages) to interrogate cancer cell features causing heterogeneity of macrophage phenotypes across a panel of NSCLCs. We measured (with 4-7 replicates for each NSCLC) mRNA expression in mouse macrophages with a panel of qPCR probes for important macrophage-related genes (Arg, Nos2, IL1beta, IL-6, CHL-3, SOCS3), and in selected cases whole-genome RNAseq; and protein expression using cytokine arrays measuring expression of 40 inflammatory cytokines. Positive controls were stimulated with LPS and IL-4. Summary of New Data: Using our platform, we characterized 70 NSCLC patient-derived lines by their ability to alter macrophage phenotype. We found: 1. the macrophage phenotypes induced by any one NSCLC were highly reproducible; 2. three major clusters of cancer polarized macrophage phenotypes: high Arg (immune suppressive), high IL-1beta (inflammatory) or high SOCS3 (cGAS-STING pathway) expression; and 3. the major oncogenotypes (KRAS, TP53, STK11, EGFR, BRAF) have no correlation to the induced macrophage phenotype. We selected 7 NSCLC “exemplar” lines representing each of these 3 clusters for RNA sequencing (mouse genes) and cytokine array protein (human) profiling. Across all clusters we found: 1. suppression of macrophage endocytosis pathways and activation of scavenger receptor A (SRA) signaling (M2 immune suppressive phenotype); and 2. increased expression of human IL6, IL8, and MCP1 proteins, which have been implicated in suppressing innate immune tumor sensing. Analyses of differences between the 3 clusters is ongoing. Conclusions: Patient-derived NSCLC preclinical models have reproducible effects on altering macrophage phenotypes in organoid cultures. Three major classes of NSCLC initiated macrophage alteration, which are not linked to oncogenotype. Cytokines secreted by the NSCLCs appear responsible for these macrophage changes, and this system provides an experimental mechanism to systematically test each as potential therapeutic targets.