Conclusions: Inhibition of RUVBL1/2 as a monotherapy has modest efficacy due to a narrow therapeutic window. This work demonstrates that RUVBL1/2 inhibitors can enhance the cancer-killing effect of radiation, but not other clinically relevant agents, specifically in tumor cells (i.e., spares normal cells). Additionally, we show that treatment with a RUVBL1/2 inhibitor can cause immune infiltration in NSCLC tumors, and a RUVBL1/2 inhibitor in combination with radiation can activate the cGAS/STING pathway. In totality, our results suggest that further research should be done looking at the efficacy of RUVBL1/2 inhibitors with immune checkpoint inhibitors, both with and without radiation, in NSCLC.

A18
Culture of Immortalized Human Alveolar Epithelial Cells in 2D and 3D to Model Lung Adenocarcinoma Progression in Vitro
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Background: Lung cancer is the leading cause of cancer death in the United States. Lung adenocarcinoma (LUAD) is the most common histologic subtype, arising from epithelial cells of terminal respiratory units called alveolus. The overall 5-year survival of lung cancer remains low at 19%. There is an urgent need to understand early events in LUAD development, as well as to develop new 1st, 2nd, and 3rd-line targeted therapies. Human organoids are powerful research tools for the molecular and mechanical manipulation of genetically diverse cells without exposing human subjects to treatment. Establishment of normal cell lines from human alveolar epithelial cells (AECs) has remained challenging due to the difficulty of growing primary cells in long-term culture. Human alveolar organoids would provide a powerful tool to 1) study LUAD development, progression, and drug resistance; 2) screen for new therapeutics; and 3) study the effects of environmental exposures on AECs. Goal: Optimize growth and genetic conditions to derive human AEC lines from purified primary cells for the stepwise modeling of LUAD. Approach: We tested different immortalization strategies using primary purified AECs to determine which condition allowed cells to continue proliferating while retaining their epithelial phenotype in two-dimensional (2D) culture and their ability to form spheroids in three-dimensional (3D) culture. Results/Discussion: Using purified primary AECs from three deceased, deidentified human subjects, we found that the initial propagation of AECs in media containing Y-27632 and subsequent transduction with Simian virus 40 Large T antigen allowed cells to divide readily in 2D as a monolayer, while expressing epithelial marker E-cadherin but not mature lung genes. When placed in 3D Matrigel culture with fibroblasts, these cells form multilobulated structures expressing mature AEC markers, reminiscent of the peripheral lung. We are currently optimizing this system to allow stepwise modeling of LUAD.

A19
Epithelial Beta 1 Integrin Regulates Lung Cancer Susceptibility Through NF-kB Signaling
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Rationale: Cell-extracellular matrix (ECM) interactions are essential for maintenance of alveolar homeostasis in the adult lung. Alveolar epithelial cells (AECs) connect to the ECM through integrins. β1 integrin is the most common lung epithelial integrin subunit, and forms the receptors for collagen, laminin, and fibronectin. We have previously shown that epithelial β1 integrin regulates AEC inflammatory signaling during alveolar homeostasis. However, the role of epithelial β1 integrin during repair after injury remains undefined. We hypothesize that epithelial β1 integrin is required for alveolar repair by regulating AEC proliferation and survival in response to injurious stimuli. Methods: We deleted β1 integrin in type 2 AECs in the adult lung in SP-C rtTA; TetO-Cre; β1f/f mice using doxycycline to induce Cre expression (called β1rtTA mice). Three-month-old β1rtTA and β1f/f mice were challenged with 3 μg/g intratracheal lipopolysaccharide (LPS) or PBS. β1f/f littermate mice and doxycycline naïve SP-C rtTA; TetO-Cre; β1f/f mice were used as controls and mice were crossed onto the CCR2-/- background. Lung slices obtained from β1rtTA and β1f/f lungs were cultured with the NF-kB inhibitor BAY-11-7082, LPS, or both. Results: We previously reported that β1-deficient type 2 AECs are inflamed in the absence of injury, exhibited increased reactive oxygen species production, increased NF-kB activation, and secreting inflammatory cytokines. Aged 2-year-old β1rtTA mice exhibited chronic progressive macropage dominant inflammation and developed adenocarcinoma. We next challenged 3-month-old β1rtTA mice with intratracheal LPS. LPS-treated β1rtTA mice had increased mortality at 7 days (43% β1rtTA vs. 91% β1f/f survival, p < .05) and an escalation in the number of recruited inflammatory cells from 24 hours to 7 days post-LPS challenge. In the β1rtTA survivors, histologic examination 21 days after LPS challenge resulted in emphysema in β1rtTA lungs, fibrotic regions identified by trichrome staining, and type 2 AEC hyperplasia. Histologic examination 6 months following LPS administration revealed sustained influx on monocyte-macrophages and early adenocarcinoma formation at 9 months of age in β1rtTA mice. Since the NF-kB pathway serves as a prosurvival mechanism, we treated ex vivo lung slices with an NF-kB inhibitor and LPS. In β1rtTA lung slices, NF-kB inhibition alone potentiated tissue remodeling and exacerbated AEC proliferation, suggesting that upregulated NF-kB plays a compensatory prosurvival role in the presence of chronically inflamed type 2 AECs. β1rtTA mice crossed to the CCR2 null background, which lack monocyte-macrophage recruitment, were protected from adenocarcinoma formation with age and following LPS challenge. Conclusions: These findings suggest that β1 integrin relies on the prosurvival properties of the NF-kB pathway to regulate AEC proliferation during homeostasis and dysregulation of β1-mediated inflammation post injury increases cancer susceptibility.

A20
Estrogen Metabolism in Patients with EGFR-Mutated and ALK-Mutated Non-Small Cell Lung Cancer (NSCLC)

Prior studies have shown that the human lung can extensively metabolize estrogen to reactive catechols. Of greatest concern is 4-hydroxyestradiol (4-OHE), a putative carcinogen, produced by cytochrome P450 1B1 (CYP1B1). In contrast, CYP1A1 metabolizes parent estrogen to 2-hydroxyestradiol (2-OHE), which are less reactive and converted to derivatives that may be antiproliferative. Data from this group strongly suggest that 4-OHE contributes to lung tumorigenesis, though its role in driver-mutated NSCLC has not been investigated. This study assessed estrogen metabolite profiles in EGFR-mutated and ALK-mutated NSCLC patients as compared to cancer-free subjects. Advanced-stage NSCLC patients with tumors that possessed either an EGFR (n = 14) or ALK (n = 8) mutation and cancer-free subjects (n = 17) were recruited from Fox Chase Cancer Center. Tumor mutation status of NSCLC patients was determined by tissue biopsy. All study participants were 50 years of age or older to circumvent any confounding influence of young age or premenopausal status on estrogen levels. NSCLC patients included 14 females and 8 males. Cancer-free subjects serving as controls were never-smoking women. Urine specimens were collected from study participants and urinary estrogen
species (E1, E2, E3, 4-OHEs, 2-OHEs, 2-OMEs) were quantified using UPLC-MS/MS. Medians were calculated and the Wilcoxon rank-sum test was used to compare estrogen metabolite measures (4-OHEs/total estrogen, 2-OHE/total estrogen, and the ratio of 4-OHEs/2-OHEs) between NSCLC patients and control subjects. EGFR-mutated NSCLC patients had a significantly higher proportion of 4-OHEs/total estrogen (0.18 vs. 0.05, p-value = 0.048) and a trend towards lower 2-OHEs/total estrogen (0.18 vs. 0.26, p-value = 0.084) as compared to cancer-free control subjects. The ratio of 4-OHEs/2-OHEs was higher in EGFR-mutated NSCLC patients as compared to cancer-free controls (0.90 vs. 0.16, p = 0.053). Differences were not seen between ALK-mutated NSCLC patients and cancer-free subjects for the measures of 4-OHE/total estrogen (0.09 vs. 0.05, p-value = 0.842), 2-OHE/total estrogen (0.20 vs. 0.26, p-value = 0.238), and the ratio of 4-OHEs/2-OHEs (0.34 vs. 0.16, p-value = 0.669). The greater relative level of 4-OHE to 2-OHE in EGFR-mutated NSCLC patients suggests that enhanced production of 4-OHE may contribute to the development of EGFR-mutated lung tumors. Targeting CYP1B1, the enzyme responsible for 4-OHE production, may be of therapeutic interest. Research is ongoing to validate these findings in a larger cohort of EGFR-mutated NSCLC patients.

A21
Targeting Glucose Reliance in Lung Squamous Cell Carcinoma
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Lung squamous cell carcinoma (LSCC) is a major class of pulmonary malignancy that accounts for 25-30% of all lung cancers. LSCC patients have benefited very little from the application of targeted therapeutic options. As a result, decades-old platinum-based chemotherapy or radiation regimens with limited efficacy and specificity remain the first-line treatment options. Therefore, identification and elucidation of targetable vulnerabilities in LSCC is urgently needed to improve therapeutic outcomes in LSCC patients. Our efforts to identify targetable pathways crucial for LSCC growth and survival led to the discovery of exceptional overexpression of glucose transporter 1 (GLUT1, encoded by SLC2A1 gene) and exceptional glucose reliance for tumor growth and survival. Mechanistically, our recently published study demonstrated that squamous lineage transcription factors, p63 and SOX2, jointly transactivate an intronic enhancer cluster in the SLC2A1 gene, and this hyperactive GLUT1-mediated glucose influx provides a carbon source to enhance the antioxidant capacity and tumorigenicity of LSCC. This previously unrecognized metabolic signature phenotypically embedded in the squamous lineage subtype of lung cancer provides a rationale to target GLUT1-mediated glucose influx. We evaluate the efficacy of ketogenic diet (dietary glucose restriction) as well as the SGLT2 inhibitor canagliflozin, an FDA-approved drug for the treatment of type 2 diabetes (pharmacologic glucose restriction), which effectivley lowers the host blood glucose levels by blocking SGLT2-mediated renal glucose reabsorption. Reduction of blood glucose lowers blood insulin levels, which effectively suppresses PI3K/AKT signaling in LSCC cells. Repurposing FDA-approved canagliflozin can be rapidly translatable as an effective therapeutic strategy for squamous cancer patients.

A22
Integrated Proteometabolomic Analysis Reveals Metabolic Vulnerabilities in Small-Cell Lung Cancer
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Small-cell lung cancer (SCLC) is the third most common histology of lung cancers and is extremely aggressive and highly metastatic. Although SCLC responds well to radiation and standard platinum-based chemotherapy, this is nearly invariably followed by relapse and the emergence of chemoresistant disease. Hence, SCLC has been declared a recalcitrant malignancy by the NCI and there is an urgent need to identify new and actionable therapeutic vulnerabilities for treatment-naive and chemoresistant SCLC. To this end we performed unbiased activity-based (ATP-binding) proteome profiling (ABPP), expression proteomics, and targeted metabolomics on a panel of SCLC and NSCLC cell lines, patient-derived lung tumor tissues, and PDX including paired treatment-naive and cisplatin-resistant SCLC. These studies revealed highly elevated activity of enzymes associated with glycolysis, lipid biosynthesis, and purine metabolism in SCLC. In addition, metabolomic analysis identified concordant upregulation of metabolites in these pathways in SCLC. We further performed screening with available metabolic drugs on SCLC and NSCLC cell lines. The results showed that the MCT1/MCT2 lactate transport inhibitor SR-13800 and the PFKFB3 inhibitors 3PO and PFK15 compromised SCLC cell growth and their combined inhibition showed synergy, provoking rapid SCLC cell death. Flux, metabolic, and genetic (CRISPR-editing) analysis of SCLC cells revealed that MCT1/2 inhibition loss blocked glycolysis and provoked a shift towards oxidative phosphorylation (OXPHOS), and that this provoked increases in intracellular lactate and dihydroxyacetone phosphate (DHAP) and a marked shift in the NAD+/NADH ratio towards NADH. In addition, levels of amino acids that can generate NAD+ were also significantly reduced. In contrast and surprisingly, PFKFB3 inhibition led to a collapse in OXPHOS and provoked increases in glycolysis and increased efflux of lactate. The combined inhibition of MCT1/2 and PFKFB3 amplified the metabolic deficits provoked by MCT1/2 and led to metabolic collapse via suppression of both glycolysis and OXPHOS. Thus, cotargeting MCT1/2 and PFKFB3 provokes synthetic lethality in SCLC, supporting the notion that their dual inhibition will be an effective treatment strategy for this lethal malignancy.

A23
A Genomically Adjusted Clinicopathologic Model Predicts Recurrence in Resected Early-Stage Lung Squamous Cell Carcinoma

Introduction: In contrast to lung adenocarcinoma, identification of clinically relevant genomic perturbations in lung squamous cell carcinoma (LUSC) remains poorly characterized. Prognostic and therapeutic decisions following surgery in early and locoregionally advanced LUSC are almost exclusively driven by the TNM classification system, omitting high-risk clinicopathologic and tumor genomic information. To address this knowledge gap, we sought to determine if a combined clinicopathologic and genomic model could predict disease-free survival (DFS) better than traditional TNM assessments in completely resected LUSC. Methods: A retrospective cohort study of a prospectively maintained database was performed for patients (N=95) with pathologic stage I-III LUSC who underwent complete resection from 2008-2018. Patients who received any induction therapy (N=9) were excluded. All patients had complete clinicopathologic data with broad-panel next-generation sequencing of the primary tumor, including matched controls to bioinformatically match germline variants. DFS, the primary endpoint, and overall survival (OS) were calculated using Kaplan-Meier. Genomic pathway alterations (N=95) were used to compare estrogen metabolite measures (4-OHEs/total estrogen, 2-OHE/total estrogen, and the ratio of 4-OHEs/2-OHEs) between NSCLC patients and control subjects. EGFR-mutated NSCLC patients had a significantly higher proportion of 4-OHEs/total estrogen (0.18 vs. 0.05, p-value = 0.048) and a trend towards lower 2-OHEs/total estrogen (0.18 vs. 0.26, p-value = 0.084) as compared to cancer-free control subjects. The ratio of 4-OHEs/2-OHEs was higher in EGFR-mutated NSCLC patients as compared to cancer-free controls (0.90 vs. 0.16, p = 0.053). Differences were not seen between ALK-mutated NSCLC patients and cancer-free subjects for the measures of 4-OHE/total estrogen (0.09 vs. 0.05, p-value = 0.842), 2-OHE/total estrogen (0.20 vs. 0.26, p-value = 0.238), and the ratio of 4-OHEs/2-OHEs (0.34 vs. 0.16, p-value = 0.669). The greater relative level of 4-OHE to 2-OHE in EGFR-mutated NSCLC patients suggests that enhanced production of 4-OHE may contribute to the development of EGFR-mutated lung tumors. Targeting CYP1B1, the enzyme responsible for 4-OHE production, may be of therapeutic interest. Research is ongoing to validate these findings in a larger cohort of EGFR-mutated NSCLC patients.