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

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 alveoli. 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
E. Plosa, J. Sucre, P. Gulleman, T. Blackwell Vanderbilt University Medical Center, Nashville, TN/US

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 macrophage 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-hydroxyestrogen (4-OHE), a putative carcinogen, produced by cytochrome P450 1B1 (CYP1B1). In contrast, CYP1A1 metabolizes parent estrogens to 2-hydroxyestrogen (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