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 controls. 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 this hypothesis.

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, it 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-naïve 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-naïve 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+ to 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.

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 treatable 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 overexpression of glucose transporter 1 (GLUT1, encoded by the 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 intrinsic enhancer cluster in the SLC2A1 gene, and this hyperactive GLUT1-mediated glucose influx provides a carbon source to enhance the antioxidative 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 effectually lowers the host blood glucose levels by blocking SGLT2-mediated renal glucose reabsorption. Reduction of blood glucose lowers blood insulin levels, which effectively suppresses P38/ERK 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


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, it 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-naïve 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-naïve 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+ to 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 Clinicoopathologic 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 clinicoopathologic and tumor genomic information. To address this knowledge gap, we sought to determine if a combined clinico-pathologic 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-Ill LUSC who underwent complete resection from 2008-2018. Patients who received any induction therapy (N=9) were excluded. All patients had complete clinico-pathologic data with broad-panel next-generation sequencing of the primary tumor, including matched controls to bioinformatically filter germline variants. DFS, the primary endpoint, and overall survival (OS) were estimated using Kaplan-Meier. Genomic pathway alterations (N=9) were
BCL6 and ARD1A genomic alterations. This exploratory genomic analysis also suggests future studies investigating these genomic alterations, further improved discrimination (CPE = 0.77).

Conclusions: We show that integration of high-risk clinicopathologic factors for lung cancer development. However, about 20% of lung cancer diagnoses are reported in individuals with no smoking history. Lung cancer in never-smokers (LCNS) is clinically distinct from tobacco-induced lung cancer, with a greater proportion of LCNS occurring in women and having adenocarcinoma histology. One of the key challenges in identifying the cancer-promoting genetic events that drive LCNS is the relatively small number of tumors that have been sequenced using genome- or exome-wide approaches. The dysregulation of receptor tyrosine kinases (RTK) has garnered plenty of interest within the cancer field, and attention has begun to turn to phosphatases regulating RTK behavior. Under normal cellular conditions, protein tyrosine phosphatases remove phosphate groups from tyrosine residues, thus maintaining signaling homeostasis. In whole-genome sequencing of primary mouse mammary tumors from the polyoma virus middle T antigen (PyMT) mouse model, we found a mutation in the protein tyrosine phosphatase receptor type H (Ptprh) gene. Targeted resequencing of 45 mouse tumors showed a conserved heterozygous or homozygous mutation present in 80% of tumors. This C>T mutation results in a valine-to-methionine shift within one of the fibronectin domains of PTPrH. Previous literature has shown interactions between PTPrH and epidermal growth factor receptor (EGFR). To determine the relevancy of PTPrH mutations in human cancer, data from The Cancer Genome Atlas (TCGA) were analyzed and revealed PTPrH mutations in 5% of non-small cell lung cancer (NSCLC) patients. Moreover, patients with a mutation in PTPrH were mutually exclusive from those with mutation or amplification of EGFR. We hypothesize a mutation in PTPrH results in a failure of PTPrH to dephosphorylate EGFR, resulting in inappropriate maintenance of downstream signaling pathways important for proliferation and evading apoptosis. Since NSCLC patients with EGFR mutations are successfully treated with tyrosine kinase inhibitors (TKI), we also hypothesize tumors with a mutation in PTPrH will be sensitive to TKIs. In support of this, we demonstrated mouse tumors with a mutation in Ptprh had increased phosphorylated EGFR (pEGFR). Furthermore, CRISPR-mediated knockout of PTPrH in H23 NSCLC cells leads to increased pEGFR. Pathway signature analysis applied to microarray gene expression data from the Breast TCGA dataset (due to low sample size in the NSCLC dataset), and single sample gene set enrichment analysis applied to RNA sequencing data from the NSCLC TCGA dataset both predicted an increase in PI3K and AKT activity. This suggested the EGFR residue targeted by PTPrH was tyrosine 1197. Western blots on Ptprh mutant mouse tumors confirmed increased levels of pAKT. Additionally, immunohistochemistry for pEGFR 1197 revealed increased staining in mouse tumors with a mutation in Ptprh, with subcellular location in the nucleus rather than the membrane. To determine whether TKIs may be an effective treatment for NSCLC patients who harbor a PTPrH mutation, H1155 and H2228 NSCLC cell lines with PTPrH mutations in the fibronectin and phosphatase domains, respectively, were subjected to a dose response curve with the TKI osimertinib. These lines show significant growth differences as compared to the negative control cell line A427. While more work is needed to elucidate the role of mutant PTPrH in NSCLC, preliminary data suggest mutant PTPrH fails to dephosphorylate EGFR, and patients with a mutation in PTPrH may benefit from TKI therapy.

The Genome-Wide Mutational Landscape of Lung Cancer in Never-Smokers: The Women’s Health Initiative (WHI) Cohort


Lung cancer is the leading cause of cancer deaths worldwide, and a positive history of smoking remains one of the most significant risk factors for lung cancer development. However, about 20% of lung cancer diagnoses are reported in individuals with no smoking history. Lung cancer in never-smokers (LCNS) is clinically distinct from tobacco-induced lung cancer, with a greater proportion of LCNS occurring in women and having adenocarcinoma histology. One of the key challenges in identifying the cancer-promoting genetic events that drive LCNS is the relatively small number of tumors that have been sequenced using genome- or exome-wide approaches. We address this gap through the collection and exome sequencing of lung tumor tissue involving 18 tumor/normal pairs identified an enrichment of somatic mutations in genes such as EGFR, KRAS, RIT1, and MET with mutations that are clinically targetable using FDA-approved or investigational agents. Overall this project will double the number of exome profiles from never-smokers and, importantly, leverage the extensive metadata curated under the WHI to evaluate secondary/environmental factors such as second-hand smoke and radon exposure and their potential role in LCNS.

Deciphering the Functional Redundancy of USP4 and USP15

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Ubiquitin-specific proteases (USPs) are a class of deubiquitinating enzymes that catalyze the removal of ubiquitin from various proteins and are involved in many cancers. Previous work established the USP paralogs USP4 and USP15 emerged from an ancestral USP about 500 million years ago from a whole genome duplication, and the majority of