uncommon binding mode to occupy both clefts to limit the impact of gatekeeper mutants but is liable to resistance of non-gatekeeper mutations.

B19
New Potential Targets of Antibody-Drug Conjugates for Small-Cell Lung Carcinoma
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Small-cell lung cancer (SCLC) remains one of the high-grade malignancies, whereas non-small cell lung cancer benefits from molecular target drugs or immune checkpoint inhibitors as a result of investigating driver mutations and immune microenvironment. However, novel driver mutation was not identified through genome-wide sequence analyses, resulting in invariable therapeutic strategy for SCLC. Therefore, we have to shift from cytotoxic agent and molecular target drug in the care of SCLC. In recent years, different approaches to hematologic malignancies and solid tumors were established in clinical situation. Antibody-drug conjugates (ADCs) is the key technique. In this study, we aimed to search new therapeutic targets for ADCs toward a paradigm shift in treatment and research of SCLC. We sought to transmembrane proteins of SCLC as new targets for ADCs with computational-biologic approach. We demonstrated 565 genes were overexpressed on 51 small-cell lung cancer cell lines compared to 30 normal tissues samples by investigating gene expression profile available in open source of Cancer Cell Line Encyclopedia and National Center for Biotechnology Information (NCBI) with Human Genome U133 Plus 2.0 Array (ThermoFisher Scientific). Among the 565 genes, 31 genes manifested increased value of compensated fluorescence signal on average by 3 or more. Of the 31 genes, by investigating RNA sequence data for normal tissue in NCBI, we identified 7 genes expressed in limited organs. We adopted these 7 selected genes as candidates for new targets of ADCs. We examined these new target genes by evaluating in vitro cytotoxicity of corresponding monoclonal antibodies followed by secondary ADCs comprising PNU-159682, a derivative of nemorubicin, using SCLC cell lines with and without overexpression of these genes. Cytotoxicity assay targeting a certain transmembrane protein, one of the candidate molecules, showed distinct effect of secondary ADC, inducing a large amount of cell death in concentration-dependent manner while secondary ADC following murine IgG isotype control exhibited lack of cytotoxicity. Secondary ADC targeting the protein showed about fourfold greater potency than that using murine IgG isotype control as a primary antibody (EC50 3.3 nM versus 13.0 nM). Conversely, CRISPR-Cas9 mediated knockout of the gene showed explicit loss of the cytotoxic effect. The expression of the gene in normal organs were examined using human total RNA, which demonstrated lower expression of the gene in normal organs. Cas9 mediated knockout of the gene showed explicit loss of the cytotoxic effect. The expression of the gene in normal organs were examined using human total RNA, which demonstrated lower expression of the gene in many organs than in brain. The distribution of the gene expression is preferable in the viewpoint of reducing side effects of the ADC, which cannot cross the blood-brain barrier. We successfully established new targets for ADCs by investigating membrane proteins and narrowing these proteins with computational-biologic approach. Through in vitro cytotoxicity assays, the protein -mediated ADC exhibited specific killing of SCLC cell lines overexpressing the gene, suggesting the gene can be a potential target of ADCs.

B20
Oncogene-Mediated ERK Signaling Suppresses Neuroendocrine Transcription Factors and Facilitates Cellular Transformation in Small-Cell Lung Cancer Through Chromatin Remodeling
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Background: In contrast to non-small cell lung cancer (NSCLC), small-cell lung cancer (SCLC) rarely harbors gene alterations that activate signaling through the receptor tyrosine kinase/RAS/RAF/MEK/ERK pathway. In addition, EGFR protein expression is universally lost during histologic transformation from mutant EGFR-driven lung adenocarcinoma (LUAD) to SCLC that occurs in a subset of patients that develop acquired resistance to EGFR tyrosine kinase inhibitors (TKIs), despite the original EGFR mutations being maintained in the transformed tumor. Based on these observations, we hypothesized that signaling through mitogen-activated protein kinases (MAPKs) is detrimental to SCLC tumors and suppresses the neuroendocrine (NE) differentiation program that is a hallmark of this lung cancer subtype. To test this, we induced MAPK signaling through expression of two LUAD driver oncogenes, KRASG12V and EGFRL858R, and assessed the impact on the phenotype and signaling profiles of SCLC. Methods: KRASG12V or EGFRL858R was exogenously expressed in an inducible manner in three SCLC cell lines (H2107, H82, and H524). Effects were characterized through microscopy, growth assays, gene expression and chromatin profiling, and Western blots of master NE transcription factors including insulinoma-associated protein 1 (INS1M1), POU class 3 homeobox 2 (BRN2), achaete-scute homologue 1 (ASCL1), and neurogenic differentiation factor 1 (NEUROD1). Results: Induction of mutant KRAS or EGFR caused transition from suspension to adherent phenotype that was reversed by pharmacologic inhibition of both ERK and AKT. Moreover, whereas both oncogenes downregulated NE transcription factors, effects were more prominent after KRASG12V induction, reflecting the difference in degrees of phospho-ERK levels. Inhibition of ERK completely rescued the repression of NE factors by KRASG12V induction, and partial effects were observed through inhibition of the downstream effectors MEK/AKT. Notably, KRASG12V-mediated suppression of NE factors was restored by inhibition of the histone modifiers p300/CBP or KDM5A in a cell line-specific manner. ATAC-seq analyses are currently underway to examine the changes of chromatin accessibility after KRASG12V induction +/- inhibition of ERK, MEK/ERK, or p300/CBP. Conclusions: In SCLC, activation of ERK and AKT by mutant KRAS or EGFR causes phenotypic transition to a NSCLC-like state, and ERK is the central hub for the regulation of NE factors. Histone modifications by hyperactivated ERK play an important role in this process and are mediated via tumor-specific mechanisms. These findings provide a biologic basis for why SCLC lacks alterations in the MAPK pathway and shed light on the underlying mechanisms of histologic version of SCLC to and from NSCLC, which may play a role in TKI resistance.

B22
Development of a Novel Serum Marker for Detecting Small-Cell Lung Cancer by Targeting a Cell Adhesion Molecule 1 (CADM1)
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Small-cell lung cancer (SCLC) accounts for about 15% of lung cancer. Although SCLC often responds favorably to combined-modality chemotherapy at the initial treatment, resistant tumors develop rapidly, which makes SCLC one of the representative cancers refractory to any therapeutic approaches. Moreover, molecular targeting therapy has not been developed for SCLC so far. Therefore, novel approaches to the diagnosis and treatment of SCLC on the basis of molecular understanding would be prerequisites to control this refractory cancer. One of the most critical issues of SCLC is its early detection in the initial screening and after chemotherapy. For this purpose, progastrin-releasing peptide (ProGRP) and neuron specific enolase (NSE) are widely used for serum markers for detection of SCLC, although combination of ProGRP and NSE can detect at most 60% of SCLC. We have
previously demonstrated that CADM1, a member of the immunoglobulin superfamily cell adhesion molecules, is highly expressed in around 75% of SCLC. In addition, SCLC expresses a splicing variant, CADM1v8/9, which is observed specifically in normal testis. Here, we report that the extracellular fragment of CADM1v8/9 is digested by ADM17 and released into cell medium or human serum. Then, we generated specific monoclonal antibody against CADM1v8/9 using Cadm1-deficient mice and developed a serum diagnostic marker for SCLC. Preliminary study shows that CADM1v8/9 detects 47% of SCLC, which is independent of and partly overlaps with the cases detected by ProGRP. CADM1v8/9 can also detect a significant portion of patients with limited disease of SCLC. Furthermore, the amount of CADM1v8/9 fragments correlates well with the disease activity of SCLC before and after the chemotherapy. These findings indicate that detection of CADM1v8/9 in serum from patients is a novel and promising approach to detect and follow up SCLC patients. CADM1 would also provide a promising target for the treatment of SCLC.

B23 Unraveling the Mechanisms of Small-Cell Lung Cancer Brain Metastasis
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Brain metastases are the most common type of intracranial tumors and are associated with high morbidity and mortality rates in cancer patients worldwide. Therapeutic options to treat brain metastases remain extremely limited, in part because of a lack of preclinical models and a limited understanding of the mechanisms allowing tumor cells from various primary sites to grow in the brain microenvironment. Small-cell lung cancer (SCLC) is a highly lethal type of lung cancer that frequently metastasizes to the brain. We have recently developed two preclinical models to investigate the interactions of SCLC cells with cells in the brain microenvironment and to identify mechanisms of SCLC brain metastasis. First, we have developed a direct intracranial transplant approach in which fluorescently labeled SCLC cells form tumors in the brain of recipient mice, including immunocompetent mice. Second, we have developed a coculture system in which SCLC cells invade brain organoids engineered from human iPS cells. Using these two models, we have found that GFAP-positive reactive astrocytes interact with SCLC cells in the brain and potentially affect the growth of SCLC brain metastases. GFAP-positive astrocytes actively infiltrate SCLC brain metastases in our preclinical models and in patients. We also show that astrocytes can promote the growth of SCLC cells in culture. Previous studies have shown that Nfib, an oncogenic transcription factor that drives the metastatic progression of SCLC, can induce expression of neuronal gene programs in metastatic SCLC cells. Neuron-astrocyte interactions play a critical role in neuronal growth and migration during development. Therefore, in an effort to determine the mechanisms underlying the interactions between SCLC cells and astrocytes, we have knocked down Nfib in SCLC cells and transplanted them into mouse brains. We found that Nfib is critical for the growth of SCLC brain metastases and that SCLC tumors with reduced Nfib expression show elevated rates of apoptosis, impaired invasion, and decreased astrocyte infiltration. Ongoing work is focusing on characterizing Nfib-downstream factors that are critical for SCLC growth and migration in the brain microenvironment, especially those with functions in neuron-astrocyte interactions.

These studies will provide better mechanistic insight into how cancer cells adapt to and grow in the brain microenvironment, which may eventually help identify new therapeutic targets to treat brain metastases.

B24 The Role of Cigarette Smoke and miR520a in Pulmonary Frizzled 9 Expression
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Lung cancer is the deadliest cancer, and for this reason treatment is highly researched. Alternatively, chemoprevention can be used to combat lung cancer in individuals who are at high risk of diagnosis, such as cigarette smokers. Frizzled 9 (Fzd9) is required in vitro for chemopreventive effects of iloprost, a prostacyclin analogue, in the lung. In non-small cell lung cancer (NSCLC) cell lines, Fzd9 activates PPARY, leading to inhibition of transformed growth. Cigarette smoke exposure decreases Fzd9 expression. The goal of this study is to elucidate the relationship between cigarette smoke and miRNA regulation of Fzd9 expression. NSCLC cells exposed to cigarette smoke condensate (CSC) showed decreased Fzd9 3' UTR activity, suggesting CSC regulates Fzd9 expression through miRNA. miRNA database analysis suggested miR-95, miR-106b, and miR-520a as potential regulators of Fzd9. Immortalized human bronchial epithelial cells (HBEC) and an Fzd9-positive NSCLC cell line (A549) transfected with miR-520a oligonucleotide mimic showed decreased Fzd9 3’UTR lucerase activity, miR-520a expression increased in HBEC and NSCLC cells after CSC exposure. We have tested a miR-520a inhibitor to use for future rescue experiments in an Fzd9-negative cell line (H322). Transient overexpression of miR-520a in HBEC and A549 did not affect cell viability or proliferation, so we made a stable miR-520a expressing HBEC line that we will use for longer-duration cell assays. miR-520a may play an important role in the regulation of Fzd9 by cigarette smoke, and future experiments will characterize this relationship and potentially impact the application of iloprost chemoprevention.

B25 Mapping the SOX2 Functional Network in Small-Cell Lung Cancer
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Small-cell lung cancer (SCLC) is a devastating and often recurring disease for which there has been little change in standard-of-care treatment over the last decade. Despite the advancement of cancer therapeutics, SCLC still has a five-year survival rate of less than 7%. By elucidating the genes and protein networks that drive SCLC tumor formation and growth, new avenues for treatment can be discovered. The transcription factor, SOX2, maintains stem cell pluripotency and is required for embryonic development. We have shown that SOX2 is a driver of SCLC. The SOX2 interactome has been studied in stem cells; however, in SCLC, the network of genes and proteins that SOX2 interacts with is still unknown. Here we present SOX2 chromatin targets as determined by chromatin immunoprecipitation (ChIP-seq) and CUT&RUN and compared binding to various epigenetic marks. The identification of SOX2 post-translational modifications suggests that they may impact its function in SCLC. Furthermore, the detection of SOX2 proximal proteins through BioID shows that SOX2 interacts with known regulators of lung cancer. As transcription factors are notoriously difficult to target therapeutically, our description of the SOX2 network presents novel targets for therapeutic development in SCLC.