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 tissue 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 four-fold 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 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 LBUAD 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 (INSM1), 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 MSK/RSK. 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 analyze the changes of chromatin accessibility after KRASG12V induction /- inhibition of ERK, MSK/RSK, 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 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 prerequisite 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...