B15  
**COP1 E3 Ligase Modulates Response to Oncogenic MAPK Pathway Inhibition**

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Oncogenic activation of the RAS-MAPK pathway drives several cancers, including a majority of non-small cell lung adenocarcinomas (LAD). RAS-MAPK pathway is activated in lung adenocarcinomas via diverse genetic alterations in upstream receptor tyrosine kinases such as EGFR and ALK as well as in RAS, BRAF, MEK, and the RAS GTPase activating protein (GAP) and tumor suppressor, NF1. Therapeutically targeting components of the RAS-MAPK pathway can lead to initial tumor responses in many patients. However, very few patients show complete responses despite harboring the targeted RAS-MAPK pathway activating genetic lesion in the tumor. Responses and hence patient survival can be improved by better characterizing the molecular basis of response and resistance to therapies targeting the RAS-MAPK pathway in lung adenocarcinomas. To identify modulators of response to MAPK pathway inhibition in lung adenocarcinomas, we conducted genetic screens in BRAF-driven human lung adenocarcinoma cells. This identified the E3 ubiquitin ligase COP1/RFWD2 as a previously unknown genetic modifier in lung adenocarcinomas. We found that depletion of COP1 and members of its protein complex, as well as proteasomal subunits, confers resistance to RAS-MAPK pathway inhibition in patient-derived lung adenocarcinoma cells with oncogenic RAS-MAPK signaling. Intriguingly, oncogenic targets of COP1 include critical MAPK pathway effectors such as ETV1. Hence, we tested if depletion of COP1 protects those MAPK pathway effectors from the impact of RAS-MAPK pathway inhibitors. COP1 depletion had a substantial impact on the levels of these effectors in the presence of RAS-MAPK small-molecule inhibitors. Upon analyzing the transcriptomic and signaling changes, we found that low levels of COP1 facilitate survival of lung adenocarcinoma cells upon inhibition of the RAS-MAPK pathway by buffering the cells from the impact of the MAPK pathway inhibitor and thereby sustaining prosurvival pathways. Additionally, depletion of COP1 in in vitro derived models of resistance also resensitized them to MAPK pathway inhibition. This study has furthered our understanding of the molecular basis of tumor cell resilience during initial treatment as well as of secondary treatment resistance. We are examining if COP1 also modulates response to MAPK pathway inhibition in vivo and if levels of COP1 could be a biomarker for predicting response to RAS-MAPK pathway inhibitor therapy in patients.

B16  
**The ROS1 Cancer Model Project: A Unique Patient-Driven Partnership to Accelerate Research**

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**Background:** ROS1 rearrangements (ROS1+) are found in a wide variety of cancer types but are relatively uncommon, occurring in 1-3% of lung, gastric, and ovarian cancers, as well as melanoma, cholangiocarcinoma, glioblastoma, and other tumor types. ROS1 has been studied primarily in lung cancer, where there are now several FDA-approved drugs to treat advanced ROS1+ lung cancer. The rarity of ROS1 fusions makes studying them more challenging, as patients are too geographically dispersed to support a traditional clinical research study. To address this challenge, the ROS1ders joined forces with a leading lung cancer advocacy organization, an international research consortium, industry, and leading academic investigators to focus efforts on this rare molecular subset of cancer. **Method:** The ROS1 Cancer Model Project currently consists of two studies supported by the Addario Lung Cancer Medical Institute’s research infrastructure and remote study capabilities. Patients are empowered to contact the study team directly and do not have to be seen at a specific site to participate in the studies and donate samples for research. Due to the sparsity of research tools available to study ROS1+ cancer, the first study focuses on creation of patient-derived xenograft (PDx) models while the second study supports creation of cell lines. The ROS1ders and GO2 Foundation for Lung Cancer have effectively utilized social media to connect with ROS1+ patients across the globe to educate them about the opportunity to participate in these ongoing research efforts. Both studies are currently open to ROS1+ patients located in North America. **Results:** The ROS1 Cancer Model Project has successfully demonstrated the feasibility and power of patient-driven research and cross-sector collaboration to implement an innovative study motivated by patient need. Since its launch, the project has effectively mobilized the international ROS1+ patient population to create new cancer models for this rare molecular subset. To date, over 30 patients have been screened, with five patients referred to the PDX study and eight patients referred to the cell line study. Together, these studies have led to the successful development of new murine and cell-line research tools and have resulted in a doubling of the preclinical models now available for ROS1 research. **Conclusion:** Through unique partnerships, the ROS1ders have accelerated the creation of new cancer models that will further researchers’ understanding of this rare molecular subset. The success of this collaboration highlights the power of patients in driving research and has laid the foundation for similar efforts by other patient groups. This effort is part of the larger Global ROS1 Initiative, which is working to address the ongoing needs of the international ROS1+ patient community.

B18  
**Structural Insight into Sensitivity and Resistance of RET Mutants to Selpercatinib (LOXO-292)**

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Selpercatinib (LOXO-292) is a RET-selective protein tyrosine kinase inhibitor (TKI) designated as breakthrough therapy by the United States Food and Drug Administration. However, structural detail of its binding to RET was elusive. Protein tyrosine kinase targeted therapies often encounter resistance due to on-target mutations. Knowledge of TKI binding and resistant mutants is important for continuous pipeline development and disease management. We have identified a panel of selpercatinib-resistant RET mutants in a preclinical model and determined the co-crystal structure of RET-selpercatinib complex to 2.06-Å resolution. Unlike vandetanib or nintedanib that insert into the gate, selpercatinib anchors one end in the front cleft and wrap around the gate wall to access the back cleft without penetrating the gate between the gatekeeper residue Val-804 and the gate wall residue Lys-758. Consequently, the gatekeeper mutants RET(V804L/M) had minimal effect on selpercatinib sensitivity. Nevertheless, among others, selpercatinib interacts with hinge and β2 residues, and its hydroxymethylpropanyl group protrudes out of solvent front. Consistently, selpercatinib-resistant mutations were found at the hinge, β2, and solvent-front residues. Our study details how selpercatinib uses an
Through Chromatin Remodeling Cell Transformation in Small-Cell Lung Cancer

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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 lung 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 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 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 estimated 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.

B29
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 lung 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 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 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 estimated 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 (H2170, 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 homebox 2 (BRN2), achaete-scute horeologue 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 examine 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 transversion 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 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