ABSTRACTS

A02 Protogenomic Characterization Reveals Therapeutic Vulnerabilities in Lung Adenocarcinoma

M.A. Gillette,1 S. Satpathy,1 S. Cao,2 S. Dhanasekaran,3 S. Vasaikar,4 K. Krug,5 F. Petralia,3 Y. Li,2 W.-W. Liang,2 B. Reva,3 R. Hong,2 S. Savage,7 G. Getz,4 Q.K. Li,8 B. Zhang,2 H. Rodriguez,2 K. Ruggles,6 A.I. Robles,1 K.C. Clauser,1 R. Govindan,2 P. Wang,5 A. Nesvizhskii,3 L. Ding,7 D.R. Mani,1 S.A. Carr1 1Broad Institute of MIT and Harvard, Cambridge, MA/US, 2Washington University in St. Louis, St. Louis, MO/USA, 3University of Michigan, Ann Arbor, MI/US, 4MD Anderson Cancer Center, Houston, TX/US, 5Icahn School of Medicine at Mt. Sinai, New York, NY/USA, 6NYU School of Medicine, New York, NY/USA, 7Baylor College of Medicine, Houston, TX/US, 8Johns Hopkins University School of Medicine, Baltimore, MD/US, 9National Cancer Institute, Bethesda, MD/US

A persistent central deficiency in our knowledge of cancer concerns how genomic changes drive the proteome and phosphoproteome to execute phenotypic characteristics. Furthermore, increasing evidence implicating epigenetic and post-translational changes in cancer biology reinforce the notion that molecular profiles based on nucleic acids are incomplete and are critically complemented by analyses of proteins and their post-translational modifications (PTMs). We present the first integrated proteogenomic study on a prospectively collected lung adenocarcinoma (LUAD) cohort, and provide new insights including on molecular taxonomy, novel mutations and fusions, protein and PTM associations with canonical driver mutations, metabolic dependencies and the immune milieu. The National Cancer Institute’s Clinical Proteomics Tumor Analysis Consortium (CPTAC) collected 110 LUAD tumors and 101 paired normal adjacent tissues using rigorous standard protocols to minimize ischemic time and other pre-analytical variability. Approximately equal numbers of Eastern (China, Vietnam) and Western patients were enrolled and the population included ~40% never-smokers. Comprehensive genomic and proteomic characterization provided whole exome, whole genome, copy number, RNAseq, microRNA, long non-coding RNA, methylation, global proteome, phosphoproteome, and acetylene data. The distribution of top driver mutations paralleled that of large genomics studies; both novel structural variants in established driver genes and novel ALK fusion partners were defined. 120 proteins including CLDN18, ANK1 and PTPRCAP had evidence of regulation by DNA methylation. Association analyses highlighted important outliers seen only in the phosphoproteinome data, including potential therapeutic targets such as SOS1 in KRAS mutant and PTPN11 (Shp2) in EGFR mutant tumors. Novel KEAP1 mutants were described including one suggesting an alternative mechanism of NEF2L2 regulation. Multi-omics clustering revealed four distinct clusters, variably enriched for place of origin, gender, and mutation status. Extensive characterization of the immune landscape of LUADs identified potential therapeutic vulnerabilities including CTLA4 and IDO1. An STK11-enriched cluster had a notably “cold” immune landscape: neutrophil degranulation was proposed as a mechanism for this immune regulation. Kinase outlier analyses suggested novel therapeutic possibilities, while tumor-normal analyses defined candidate diagnostic biomarkers, cancer testis antigens and other neoantigens, and helped illuminate carcinogenesis. These and other analyses are intended to provide new insights into LUAD biology and facilitate testable therapeutic hypotheses, including for the development of targeted chemo- or immuno-therapies. Furthermore, this diverse, densely characterized and closely annotated sample population provides a vast dataset that should be an important resource for the lung cancer and broader scientific communities.

A03 Lung Adenocarcinoma Resident Microbiome May Contribute to Cancer Hypomethylation Status

E.A. Marshall,1 E.A. Vucic,2 F.S.I. Filho,3 J.M. Leung,2 S. Lam,1 W.L. Lam1 1BC Cancer Research Centre, Vancouver, BC/CA, 2New York University School of Medicine, New York, NY/US, 3UBC Centre for Heart Lung Innovation, Vancouver, BC/CA

Lung cancer is a devastating disease, and is responsible for the greatest fraction of cancer-associated deaths worldwide. Human lungs were long thought to be sterile, but as a barrier organ, are colonized by numerous bacterial communities. Here, we sought to characterize the lung adenocarcinoma (LUAD) microbiome and determine if it plays a role in tumor behaviour. After patient consent, paired LUAD tumors and adjacent non-malignant tissues (NM, n=77) were obtained. Extracted DNA was sequenced (165 rRNA V4 regions) using MiSeq. Methylation status of tumor tissue was determined by DNA bisulfite conversion and hybridization to the Illumina Human Methylation 27 array after tissue microdissection and DNA extraction. Methylation data was normalized, and average Beta values were compared by paired T-test. Validation of bacterial abundances was performed on publicly available whole RNA sequencing data depleted of reads aligning to the human genome (TCGA, 484 tumors and 58 NM). The potential functionality of the bacterial metagenome was assessed using the PICRUSt2 platform. When LUAD tumors are compared to NM tissue, we observe an increase in alphaproteobacteria, specifically Bradyrhizobium (p-adjusted=0.002). Conversely, a significantly lower abundance of gammaproteobacteria (Acinetobacter) is observed in the tumors, and an enrichment of this family is observed in NM samples (p-adjusted=0.03, Figure 1). Interestingly, we also observed a significant increase in Deinococcus in tumors (p=0.04; previously reported in LUSC). Using functional metagenome prediction, we observed a significant decrease in S-adenosyl-L-methionine synthesis (SAM; a global methyl donor) when tumors were compared to NM samples. In assessing the global patterns of DNA methylation in corresponding tissues, we observed hypomethylation of tumors compared to NM tissue genome-wide (p<0.001). To delineate the association of bacterial profiles with observed methylation patterns, we assessed tumor methylation data in the context of predicted SAM involvement. Indeed, tumors with high predicted SAM biogenesis in their microbiome had significantly more methylated regions than those with low involvement (high/low quartiles, p=0.002). Here, we assess the microbiome profile of LUAD and NM tissue, and find that LUAD is enriched in alphaproteobacteria and deficient in gammaproteobacteria. In tumors, we find that down-regulation of SAM biogenesis in the bacterial population, potentially as a result of intratumoral selection pressure, is associated with patterns of global hypomethylation in lung cancer.

A04 Lung-Resident Microbial Signature Precedes Signs of Lung Malignancy

E.A. Marshall,1 F.S.I. Filho,2 D.D. Sin,1 S. Lam,1 J.M. Leung,2 W.L. Lam1 1BC Cancer Research Centre, Vancouver, BC/CA, 2UBC Centre for Heart Lung Innovation, Vancouver, BC/CA

Shifts in the microbial populations that colonize human tissues have been shown to affect host biologic pathways. In fact, changes in the lung-epithelial-resident microbiota have been associated with various lung diseases. In cancers in general, specific bacteria have been shown to confer increased risk of disease (e.g., H. pylori in gastric cancer). In
lung cancer, the tumor microbiome has been shown to be less diverse than normal tissue, but the effect of microbial composition alterations in airways prior to diagnosis of lung cancer is unknown. We sought to characterize the microbiome in airways of patients found to have lung cancer on follow-up. Following consent, bronchial brushes were obtained from 48 patients at a high risk of lung cancer. With a mean follow-up of 9.4±1.2 years, 5/48 were diagnosed with lung cancer, and 3/48 were diagnosed with lung cancer at bronchoscopy. 16S sequencing was performed on bronchial epithelial taken from the airways of each patient, and the QIIME2 platform was used to classify the bacterial populations. The bacterial taxonomy, alpha, and beta diversity measures were compared according to cancer status, and bacterial metagenome functionality was assessed using PICRUSt2. We found that patients with lung cancer and those who would develop it had lower airway bacterial diversity. Further, individuals who developed lung cancer had significantly lower bacterial populations. The bacterial taxonomy, alpha, and beta diversity measures were compared according to cancer status, and bacterial metagenome functionality was assessed using PICRUSt2. We found that patients with lung cancer and those who would develop it had lower airway bacterial diversity. Further, individuals who developed lung cancer over time displayed significantly different airway microbiome profiles from those who did not, but similar profiles to those who already had cancer (p<0.0001). With global taxonomic shifts observable at the phylum level. Using gene content inference, we observed that the lung-resident bacterial communities of patients with prevalent and incident cancers had significantly different metabolic profiles when compared to patients with no cancer. In particular, we observed an enrichment in the metabolites associated with cancer pathway (Wnt and Notch) activation (p-adjust<0.0001), implicating a role of lung-resident bacterial communities in cancer initiation. Validation in an independent cohort consisting of 55 incident cancer, 18 prevalent cancer, and 263 noncancer subjects is ongoing. Here, we profile the microbial community resident to the lung epithelium, and detect changes in this community years prior to the clinical detection of lung cancer. This work lays a foundation for further prospective studies leveraging microbiome profiles to further our understanding of the role of the lung microbiome in the pathogenesis of lung cancer.

A05 ART1, a Mono-ADP-Ribosyltransferase, Regulates Tumor-Infiltrating CD8+ T Cells and Is Highly Expressed in EGFR Mutated Lung Cancers

S. Mukherjee1, E. Wennerberg1, C. Hung2, N. Saadallah2, S. Kariyawasam1, M.K. Hussein1, N. Narula1, P. Adusumilli3, A. Borczuk4, N. Altorki5, T. McGraw6, B.M. Stiles1
1Weill Cornell Medicine, New York, NY/US, 2Central Michigan University College of Medicine, Mt. Pleasant, MI/US, 3New York University, New York, NY/US, 4Memorial Sloan Kettering Cancer Center, New York, NY/US

Introduction: ADP-ribosyltransferase 1 (ART1), a GPI-linked cell surface protein, is broadly expressed at the protein level in human tumors and has been linked to tumor progression in colon cancer and gliomas. ART1 may regulate the immune microenvironment through mono-ADP-ribosylation of the P2X7 receptor on CD8+ T cells, leading to T-cell apoptosis through NAD-induced cell death. P2X7R expression is prominent on tissue resident memory (Trm) CD8+ T cells, which have increasingly been recognized for their critical role in immune response. We evaluated the role of ART1 in an immune-competent murine model and sought to determine the expression of ART1 in human tumors, particularly EGFR mutated tumors, which are known to be poorly responsive to immunotherapy. Methods: Initial experiments were performed using the KP1 lung cancer cell line derived from lung tumors of KRASG12D/P53-/- mice. KP1-shART1 cells with doxycycline (DOX)-inducible knockdown of ART1 were generated by lentiviral constructs and used in flank and tail vein tumor models to determine ART1 effects on tumor growth. A lung adenocarcinoma tissue microarray (TMA) was then trained and scored for ART1 expression in order to determine ART1 expression in human tumors. We also evaluated ART1 expression in patient samples by whole-tumor RNAseq and IHC from a prospective clinical trial of neoadjuvant durvalumab +/- sub- ablative radiation therapy (NCT02904954). Results: ART1 knockdown significantly decreased tumor burden in flank and tail vein tumor models. Populations of tumor-infiltrating CD8+ T cells and of P2X7R+/CD8+ T cells were higher with ART1 knockdown, suggesting that ART1 expression on tumor cells may regulate tumor-infiltrating T cells. In human lung cell lines, the EGFR+ cell line H1650 expressed significantly more cell surface ART1 than A549 cells or BEAS cells (2.7- and 6.9-fold, respectively). In the TMA, among 463 stage I patients, 257 patient tumors (55.5%) strongly expressed ART1. Among patients with EGFR mutated tumors (n=79), 69.6% strongly expressed ART1 compared to 52.9% of KRAS+ tumors (n=119, p=0.03). In NCT02904954, among patients with whole-tumor RNAseq performed from preoperative biopsy (n=21), relative ART1 expression was 3-fold higher in EGFR+ patients (n=6). Median post-treatment H-scores for ART1 staining in resected tumors were also higher in EGFR+ tumors (120 vs. 77.5, p<0.0065). No patients with EGFR+ tumors (n=8) had a major pathologic response to neoadjuvant durvalumab +/- RT, compared to a 38% combined arm MPR rate in EGFR- tumors (n=34). P2RX7 was strongly expressed in post-treatment tumors by whole-tumor RNAseq and trended higher in responders. Conclusions: ART1 expression on lung cancer cells modulates tumor-infiltrating CD8 T cells. Knockdown of ART1 abrogates tumor growth, suggesting that ART1 may be a potential novel immune checkpoint and a therapeutic target. ART1 is particularly overexpressed in EGFR mutated lung cancers and may provide one mechanism to help explain their poor response to immunotherapy.

A06 Tri-complex Inhibitors of the Oncogenic, GTP-Bound Form of KRASG12C Overcome RTK-Mediated Escape Mechanisms and Drive Tumor Regressions in Preclinical Models of NSCLC


RAS proteins are small GTPases that drive cell proliferation and survival when bound to GTP. Mutant RAS proteins are found in approximately one third of NSCLC, and exist predominantly in the GTP-bound state, leading to aberrant downstream signaling via interaction with effectors such as RAF. Recently, multiple potent, covalent inhibitors of the oncogenic mutant KRASG12C have entered development and are driving high lung cancer response rates in early clinical trials. These inhibitors target the inactive, GDP-bound form of KRASG12C, driving high lung cancer response rates in early clinical trials. These inhibitors target the inactive, GDP-bound form of KRASG12C, KRASG12C(Off), and thus rely on the residual intrinsic hydrolysis of GTP to cycle KRASG12C proteins through the GDP-bound state. This mechanism is vulnerable to adaptive responses in cancer cells that increase upstream signaling to further elevate the relative abundance of the active, GTP-bound state, KRASG12C(ON). An inhibitor that directly targets KRASG12C(ON) would overcome this limitation. We have developed tri-complex inhibitors of KRASG12C(ON) that promote a ternary complex between KRASG12C and the immunophilin cyclophilin A (CypA). KRASG12C(ON) inhibitors attenuate both RAS-MAPK signaling and cell viability in cancer cell lines bearing KRASG12C mutations. In vivo administration of a KRASG12C(ON) inhibitor drives dose-dependent tumor regressions in the NCI-H358 KRASG12C NSCLC xenograft mouse model and is well tolerated. Consistent with targeting the KRAS(ON) and its inhibitory activity in vitro is unaffected by RTK activation, whereas the activity of first-generation KRASG12C(Off) inhibitors is significantly attenuated. In addition, proliferation of NCI-H358 cells in vitro is suppressed for a significantly longer duration with KRASG12C(ON) inhibitor treatment compared to KRASG12C(Off) inhibitors. The ability to target the GTP-bound form of mutant KRAS permits a broad array of combination opportunities. Combination of