ATX/LPA UPREGULATION VIA MICRORNA-29 LOSS SUPPRESSES CYTOTOXIC T CELLS AND PROMOTES RESISTANCE TO ANTI-PD-1 IN NON-SMALL CELL LUNG CANCER

**Background** Immune checkpoint inhibitors (ICI) have revolutionized oncological treatment strategies for many tumor types, including non-small cell lung cancer (NSCLC), in which response rates are ~35% in patients harboring KRAS and TP53 (KP) mutations. While promising, most patients demonstrate no response or develop resistance while on treatment, underscoring the need to better define suppressive mechanisms in the tumor-immune microenvironment. Experimental murine models of KP lung cancer demonstrate upfront sensitivity to PD-1 blockade but rapidly acquire resistance, providing useful tools to analyze tumor-intrinsic resistance mechanisms.

**Methods** We analyzed transcriptome data from anti-PD-L1 treated KP tumors and identified differentially expressed genes between response and resistance timepoints. Additionally, we performed multiparameter flow cytometry to define the tumor-immune microenvironment with manipulated expression of candidate gene expression. We also utilized syngeneic and autochthonous models of KP lung cancer, as well as patient-derived organoids (PDOs), to test the efficacy of targeted agents alone and with anti-PD-1 on tumor growth and metastasis.

**Results** Our data identified autotaxin (ATX) and the metabolite it generates, lysophosphatidic acid (LPA), to be highly expressed in ICI resistant tumors. Conversely, the predicted post-transcriptional regulator of ATX, miR-29, was downregulated in resistant tumors. Analysis of TCGA lung adenocarcinoma (LUAD) patient datasets revealed a significant negative correlation between miR-29 and ATX, whereas re-expression of miR-29 in tumor cells directly repressed ATX expression via binding to the 3'UTR, providing evidence that miR-29 is a bona fide ATX regulator. To link ATX with immune functionality, we correlated ATX expression in the LUAD dataset with an immune gene signature encompassing immune checkpoints and suppressive molecules. These data revealed ATX positively correlated with many suppressive molecules, including PD-L1, PD-1 and TIM-3. To further understand the mechanism by which ATX/LPA contributes to immunosuppression, we performed flow cytometric analyses on isogenic tumors pairs with manipulated ATX expression, finding that its expression inversely correlated with CD8+ T cell proliferation and cytotoxic functionality. Similarly, exogenous expression of LPA directly diminished effector T cell cytokine secretion and differentiation. Lastly, co-targeting ATX or the downstream LPAR5 receptor with anti-PD-1 in vivo and in PDOs embedded in a 3-D microenvironment significantly inhibited tumor progression.

**Conclusions** Our data indicate that miR-29 loss drives aberrant ATX/LPA expression in tumors with ICI resistance. Overexpression of this axis in turn diminishes cytolytic functions of CD8+ T cells via LPAR5 signaling. These results provide evidence that this axis acts as an immunosuppressive checkpoint, providing rationale that co-targeting it with ICI should improve anti-tumor immune responses.

**Ethics Approval** All animal studies were completed under the approval of the University of Texas MD Anderson Cancer Center Institutional Animal Care and Use Committee (IACUC) (protocol #1271) or the Emory University IACUC (protocol #20170322). Lung cancer patient specimens were collected under the following protocols: IRB0009857 Lung and Thoracic Malignancies Satellite Bank, IRB00045796 Storage & Research Use of Tissue & Information, IRB00095411 Winship Discovery/Total Cancer Care biorepository at the Winship Cancer Institute of Emory University (Winship), and IRB00098377: Molecular Analysis of Emory Lung Cancer Specimens. All specimens and data in this study were collected under the authority of established biorepository protocols. Participants in this study sign IRB-approved informed consent forms as part of the biorepository studies.

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