**Loss of miR-29 as a mechanism of diminished anti-PD-1 response in lung cancer**

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Immunosuppressive checkpoint inhibitors (ICI), like those that block the PD-1/PD-L1 axis, have revolutionized oncological therapy, including for patients with non-small cell lung cancer (NSCLC). Clinical studies demonstrated objective response rates in 28-40% of advanced stage NSCLC patients depending on PD-L1 status. While encouraging, these data indicate that most patients demonstrate no clinical benefit or rapidly acquire resistance even in PD-L1-high populations. Therefore, there remains a critical need to dissect key tumor cell survival dependencies to overcome treatment resistance. To discover tumor-driven mechanisms of anti-PD-1 resistance, we performed single cell RNA-sequencing on KRAS/p53 mutant murine lung tumors with sensitivity or resistance to anti-PD-1 (PD1S or PD1R, respectively) and focused on predicted upstream regulators of differentially expressed genes (DEGs) in PD1R tumors, specifically within the malignant cell cluster. We found that the micro-RNA miR-29 was strongly predicted to be a post-transcriptional suppressive molecule of numerous DEGs in the dataset that were upregulated with resistance. Thus, we hypothesized that miR-29 downregulation associated with anti-PD-1 resistance causes vast tumor cell intrinsic and extrinsic effects on the microenvironment that diminish anti-tumor immune responses. Analysis of genes that correlate with miR-29 expression in TCGA lung adenocarcinoma datasets revealed positive enrichment in immune-related pathways, including adaptive immune response and T cell activation, by gene set enrichment analysis. Irrespective of treatment, patients with low miR-29 expression have significantly shortened overall survival compared to miR-29-high patients. In the PD1R murine models, we confirmed miR-29 downregulation compared to PD1S models and probed for several predicted/known miR-29 target genes. Specifically, the PD1R models had significantly increased expression of several immunosuppressive molecules including *Enpp2*/ATX (as published recently by our group) and *Cd276*/B7-H3, and genes involved in tumor:extracellular matrix (ECM) interactions and metastasis like *Lamc1*, *Itgb1,* and *Snail1*. Re-expression of miR-29 in PD1R cells was sufficient to downregulate expression of these genes and importantly, reinvigorate CD8+ T cell proliferation while decreasing expression of exhaustion markers PD-1 and LAG-3. Together, our data provide evidence that the miR-29 axis serves as a regulator of a vast transcriptional network that functions to promote anti-tumor immunity and ICI response in lung cancer.