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HEDGEHOG SIGNALING DRIVES EPITHELIAL-TO-MESENCHYMAL TRANSITION, IMMUNE EVASION, AND ANTI-PD-1 RESISTANCE THROUGH COORDINATED UPREGULATION OF WNT LIGANDS AND PGE2 SYNTHESIS

¹Nicholas DeVito*, ²Michael Sturdivant, ¹Balamayooran Theivanthiran, ¹Y-Van Nguyen, ¹Michael Plebanek, ¹Brent Hanks. ¹Duke University Medical Center, Durham, NC, USA; ²University of North Carolina, Chapel Hill, NC, USA

Background Immunotherapy resistance has been correlated with epithelial-to-mesenchymal transition (EMT),^{1, 2} however our understanding of tumor-intrinsic mechanisms driving this immune evasive phenotype is lacking. We have previously shown that Wnt ligands are upregulated in anti-PD-1 resistant melanomas,³ and postulated that upstream transcriptional regulation of select EMT pathways may underpin these findings. The hedgehog signaling (HH) transcription factor Gli2 promotes EMT.

Methods Gli2 was constitutively activated (Gli2^{CA}) in a BRAF^{V600E}PTEN^{-/-} murine cell line via an N-terminal truncating mutation and silenced using CRISPR-Cas9. Multi-parameter flow cytometry and RNAseq was utilized to evaluate the impact of Gli2 on the tumor immune microenvironment. Anti-PD-1 resistance studies were performed in Gli2^{CA} and control tumors. Bioinformatics studies were conducted using the melanoma TCGA and Hugo et al databases.²

Results We found upregulation of Gli2 targets in patients with anti-PD-1-refractory metastatic melanoma as well as in an autochthonous BRAF^{V600E}PTEN^{-/-} melanoma model after escape from anti-PD-1. RNAseq and Western blot studies demonstrated Gli2^{CA} to promote EMT and Wnt ligand production in addition to upregulated COX2 in BRAF^{V600E}PTEN^{-/-} melanoma. This finding was reversed by genetic ablation and pharmacologic inhibition of Gli2, implicating a previously undescribed role for Gli2 in modulating COX2. These data were consistent with a notable correlation between a Gli2 signature and a prostaglandin synthesis signature in human melanoma TCGA database. Flow cytometry analysis showed exclusion of cytolytic T and NK cells, a shift from cDC1s to cDC2s, and enhanced MDSC recruitment in Gli2^{CA} tumors. Consistent with these findings, whole tumor RNAseq of Gli2CA tumors demonstrated a decrease in *Cd3e*, *Prf1*, and *Xcr1* with a concomitant increase in *Cxcl1*, *Cxcl2*, *Ccl2*, *Ptgs2*, and *Arg1* relative to control tumors. RNAseq of FACS-sorted DCs from Gli2^{CA} tumors demonstrated a loss of cDC1-associated genes including *Xcr1*, *Wdfy4*, and *Clec9a* compared to DCs derived from control tumors. In-line with our previous results showing that Wnt5a promotes MDSC recruitment in a Yap-dependent manner,⁴ we found that Yap inhibition or Wnt5a deletion in the BRAF^{V600E}PTEN^{-/-}Gli2^{CA} cell line diminished MDSC-recruiting chemokines. Further consistent with these findings, Gli2^{CA} tumors resist anti-PD-1 antibody therapy.

Conclusions Our data demonstrates that the HH transcription factor Gli2 drives the development of a tolerogenic tumor microenvironment unfavorable to anti-PD-1 immunotherapy by coordinating the upregulation of Wnt ligand expression and prostaglandin synthesis (figure 1). We propose that HH gene signatures are worthy of further study as a guide for selecting Wnt ligand and prostaglandin inhibitors in future immunotherapy studies.

Abstract 923 Figure 1 Gli2 in tumors promotes Wnt and prostaglandin signaling, generating an immunosuppressive microenvironment

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