**Background**

Immunotherapy resistance has been correlated with epithelial-to-mesenchymal transition (EMT), 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, 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 (Gli2CA) in a BRAFV600E/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 Gli2CA and control tumors. Bioinformatics studies were conducted using the melanoma TCGA and Hugo et al databases.

**Results**

We found upregulation of Gli2 targets in patients with anti-PD-1-refractory metastatic melanoma as well as in an autochthonous BRAFV600E/PTEN−/− melanoma model after escape from anti-PD-1. RNAseq and Western blot studies demonstrated Gli2CA to promote EMT and Wnt ligand production in addition to upregulated COX2 in BRAFV600E/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 Gli2CA 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 FAC-sorted DCs from Gli2CA tumors demonstrated a loss of cDC1-associated genes including Xcr1, Wdly4, 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, we found that Yap inhibition or Wnt5a deletion in the BRAFV600E/PTEN−/−Gli2CA cell line diminished MDSC-recruiting chemokines. Further consistent with these findings, Gli2CA 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.

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**REFERENCES**


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