Background Immunotherapeutic resistance correlates with mesenchymal transformation (MT), however we are unable to therapeutically target this immune evasive phenotype. We have shown that in anti-PD-1 resistant melanomas, the Hedgehog (Hh) transcription factor Gli2 drives the upregulation of Wnt and prostaglandin synthesis, which promotes the recruitment of granulocytic myeloid-derived suppressor cells (PMN-MDSCs) leading to immune evasion and immunotherapy failure. Hh activation has been described in immunotherapy resistance, however targeting has focused on SMO inhibition.

Here, we demonstrate the utility of a Gli2 signature to predict anti-PD-1 immunotherapy failure and determine whether selective prostaglandin receptor inhibition (EP2i/EP4i) can overcome this resistance pathway in melanoma.

Methods Gli2 was constitutively activated (Gli2CA) in a BRAFV600E/PTEN-/- melanoma cell line by inducing an N-terminal truncating mutation. Multi-parameter flow cytometry, RNaseq, and scRNAseq were utilized to evaluate the impact of Gli2 activity and therapeutic treatments on the tumor immune microenvironment. Anti-PD-1, EP2i/EP4i, PORCNi (Wnt ligand inhibition), and PMN-MDSC ablation in vivo studies were performed in Gli2CA and control tumors. Nanostriing analysis was performed on clinical melanoma tumor specimens and bioinformatics studies were conducted using the TCGA and Hugo et al databases.

Results Gli2CA tumors display resistance to anti-PD-1, where flow cytometry revealed persistent exclusion of CD103+ antigen-presenting DCs, activated T and NK cells, and greater numbers of PMN-MDSCs compared to control tumors. Gli2 upregulates genes involved in prostaglandin synthesis and PGE2 production, which is ablated in Gli2-knockout melanomas. Treatment with EP2i/EP4i suppresses tumor growth, increasing levels of CD8+ T cells, NK cells, and cDC1s in Gli2CA but not in control tumors, and this is further potentiated by anti-PD-1. EP2i/EP4i markedly reduces PMN-MDSCs in Gli2CA tumors, and anti-Ly6G depletion of PMN-MDSCs had a similar impact on the immune microenvironment. scRNAseq showed enhanced NK cell activity with EP2i/EP4i over PORCNi in Gli2CA tumors, and while both therapies reduced PMN-MDSC numbers, EP2i/EP4i also diminished suppressive markers. Gli2 and prostaglandin signatures correlate in the melanoma and colorectal TCGA. Importantly, a Gli2 signature predicts for anti-PD-1 resistance in a subset of melanoma patients.

Conclusions Gli2 drives the development of a tolerogenic tumor microenvironment during MT via Wnt and prostaglandin signaling, leading to anti-PD-1 failure through enhanced PMN-MDSC recruitment and activity. This state can be reversed with EP2i/EP4i in mouse models. These results suggest that a Gli2 signature may predict for EP2i/EP4i sensitivity in a subset of anti-PD-1 resistant melanomas.

REFERENCES


