Epigenetic modulation of myeloid derived suppressor cells decreases suppressive signaling through the STAT3 pathway

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INTRODUCTION

Immune checkpoint inhibitors (ICIs) have shown promise in managing various cancers and improving patient quality of life. However, patients with metastatic breast cancer are considered intrinsically or eventually resistant. A proposed mechanism of resistance to ICIs is the extensive infiltration of the tumor microenvironment (TME) with immunosuppressive cells, such as myeloid derived suppressor cells (MDSCs). Our group and others have investigated MDSCs as a therapeutic target to sensitize the TME to ICIs. We have reported that epigenetic reprogramming of MDSCs by inhibiting histone deacetylases (HDACs) 1 and 3 with Entinostat decreases MDSC immunosuppressive function and contributes to ICI response[1,2]. However, a clear molecular mechanism of HDAC1/3 inhibition driving ICI response has yet to be understood. In breast intratumoral MDSCs, breast-to-lung metastatic MDSCs, and an MDSC-like cell line, we found that Entinostat decreases STAT3 phosphorylation. We hypothesized that Entinostat decreases MDSC suppression through the STAT3 pathway.

METHODS

Using the syngeneic NT2.5-LM NeuN mouse model of metastatic breast cancer[3], we established spontaneous lung metastases and treated with vehicle or Entinostat (5 mg/kg by oral gavage, 5x /week) for 3 weeks. Single cell RNA sequencing (scRNAseq) was performed on macro-dissected lung metastases.

Using bead-isolated intratumoral granulocytic-MDSCs (G-MDSCs) from lung metastases, we performed western blot to probe for protein expression levels.

Using J774M cells, an MDSC-like cell line, we performed ChIP-seq using STAT3 pulldown to identify STAT3 targets that Entinostat regulates. Sequences were aligned to mm10 murine genome. Peaks were called with MACS2 and visualized with IGV. Bulk-RNA seq was performed on Entinostat treated cells.

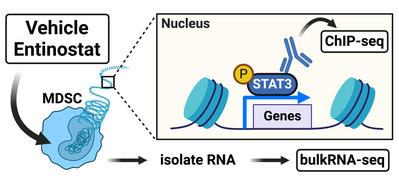

Fig. 1: Epigenetic modulation decreases STAT3 phosphorylation in metastatic TME MDSCs

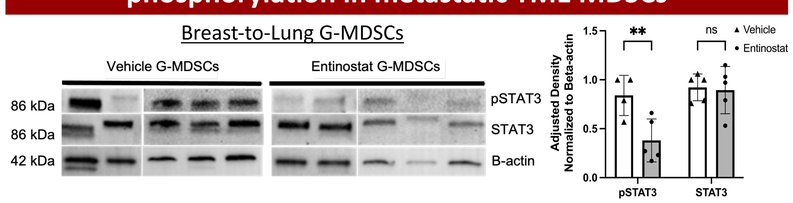

Fig. 1: Epigenetic modulation decreases pSTAT3 in MDSCs from mice. Quantified western blots of isolated MDSCs from breast-to-lung metastases in 5 mice treated with vehicle or Entinostat reveals decreased pSTAT3 with Entinostat treatment. Each column is one mouse. Quantifications are adjusted density normalized to beta-actin. **p<0.01.

Fig. 2: Epigenetic modulation decreases STAT3 enrichment at promoters of suppression related genes in MDSCs

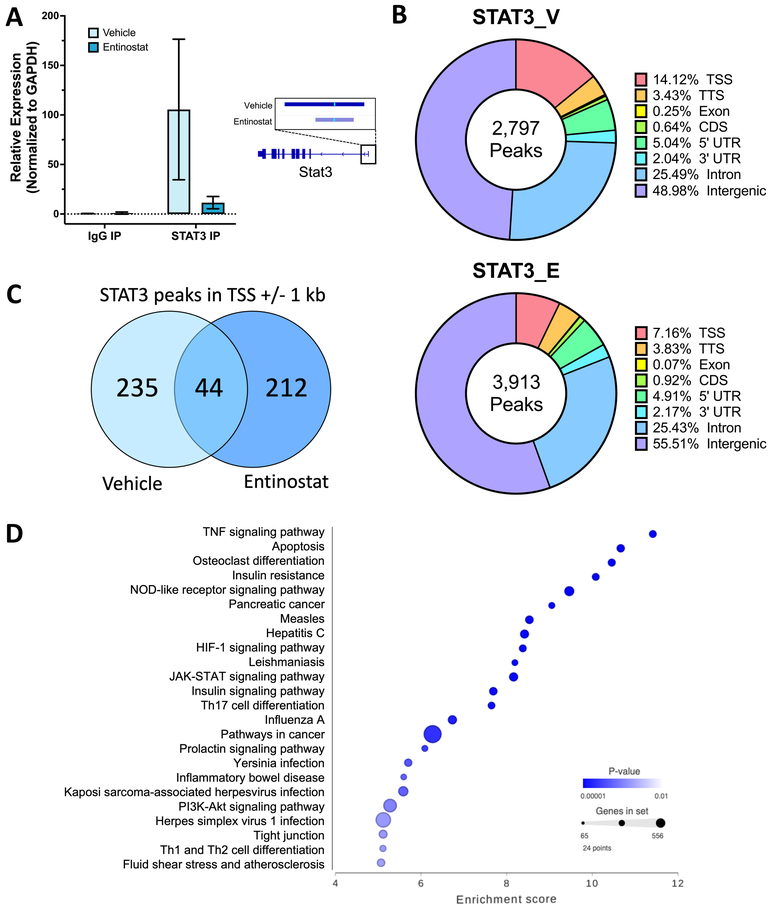

Fig. 2: Epigenetic modulation decreases STAT3 enrichment at promoters of suppressive genes in MDSCs. A. ChIP-qPCR of IgG- vs. STAT3-IP genomic DNA at *Stat3* promoter, normalized to *Gapdh* promoter, showing positive enrichment that decreases with Entinostat. ChIP-seq NarrowPeak shows decreased STAT3 enrichment at *Stat3* promoter with Entinostat. **B.** ChIP-seq peak distribution from vehicle and Entinostat treated STAT3-IP. **C.** Entinostat decreases STAT3 peaks in promoters, TSS +/- 1 kb. **D.** Pathways analysis of vehicle and Entinostat overlapping peaks. **E.** ChIP-seq NarrowPeaks show decreased STAT3 enrichment at MDSC suppressive genes.

Fig. 3: Epigenetic modulation decreases STAT3 upstream receptors and increases STAT3 negative regulator in MDSCs

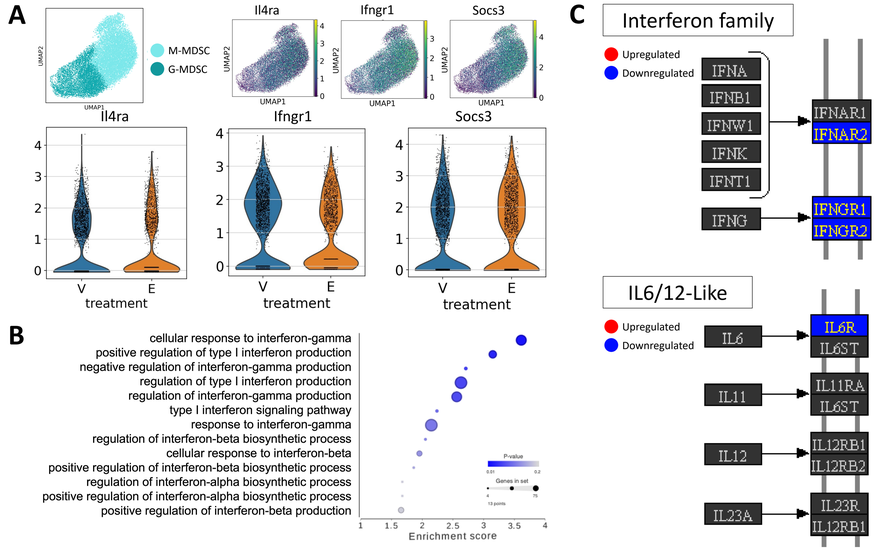

Fig 3: Epigenetic modulation decreases expression of STAT3 upstream receptors and increases expression of STAT3 negative regulator in MDSCs. A. UMAP of MDSC cluster from scRNAseq of murine NT2.5-LM breast-to-lung metastases with gene expression distribution and violin plots showing decrease in *Il4ra* and *Ifngr1* and increase in *Socs3* with Entinostat ($p<0.01$). **B.** Gene set enrichment analysis of J774M bulkRNA-seq, filtered for interferon signaling.[2] **C.** Cytokine-cytokine receptor interactions from DEseq2 in J774M showing decreased expression of *Ifnar2, Ifngr1, Ifngr2,* and *Il6r* with Entinostat (fold > 2).

CONCLUSIONS

Our findings provide new evidence implicating a STAT3-mediated mechanism leading to decreased MDSC suppressive signaling upon epigenetic regulation and histone deacetylase inhibition with Entinostat. These findings and planned studies may lead to a more complete understanding of the mechanism driving response in the combination therapy of Entinostat + ICIs in clinical trials[4], potentially informing development of more specific therapeutics for future preclinical studies and identification of biomarkers for clinical screening.

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