HDAC1 and HDAC3 inhibition decreases myeloid derived suppressor cell function through STAT3-mediated apoptosis

Aaron G. Baugh[1], Edgar Gonzalez[1], Sabrina K. Zhong[1], Matthew B. Jacobo[1], Kaliya Acevedo[1], Jesse Kreger[2], Yingtong Liu[2], Adam L. MacLean[2], Evanthia T. Roussos Torres[1]

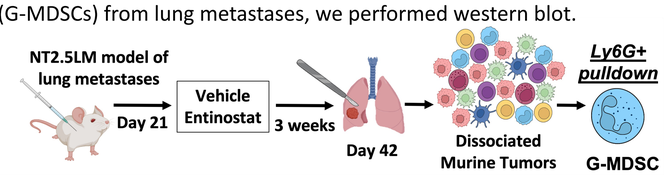
[1] Keck School of Medicine of USC, Los Angeles, CA [2] University of Southern California, Los Angeles, CA

INTRODUCTION

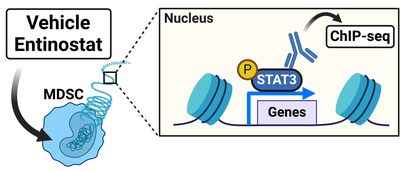
Myeloid derived suppressor cells (MDSCs) are key players in an immune-suppressed tumor microenvironment (TME) and significantly contribute to immune checkpoint inhibition (ICI) resistance in metastatic breast cancer, making them favorable targets for cancer immunotherapy. Our group and others have investigated MDSCs as therapeutic targets to sensitize the TME to ICIs. We have reported that epigenetic modulation of MDSCs by inhibiting histone deacetylases (HDACs) 1 and 3 with Entinostat decreases 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, a transcription factor known to play a role in MDSC suppression of T cells. We hypothesized that Entinostat decreases MDSC suppression through modulation of the STAT3 pathway.

METHODS

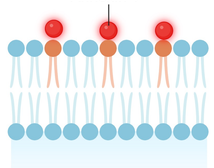
Using the syngeneic NT2.5-LM NeuN mouse model of metastatic breast cancer[3], we established spontaneous lung metastases and treated with vehicle or Entinostat (5 mg/kg by oral gavage, 5x /week) for 3 weeks. Using bead-isolated intratumoral granulocytic-MDSCs (G-MDSCs) from lung metastases, we performed western blot.



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.



To measure apoptosis, J774M cells treated with Entinostat and STAT3 inhibitors were stained for Annexin V and Propidium Iodide.



HDAC inhibition decreases STAT3 phosphorylation in metastatic TME MDSCs and MDSC-like cell line J774M

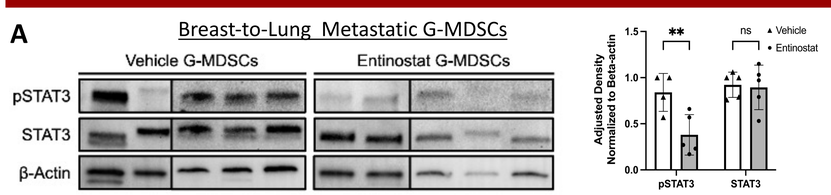
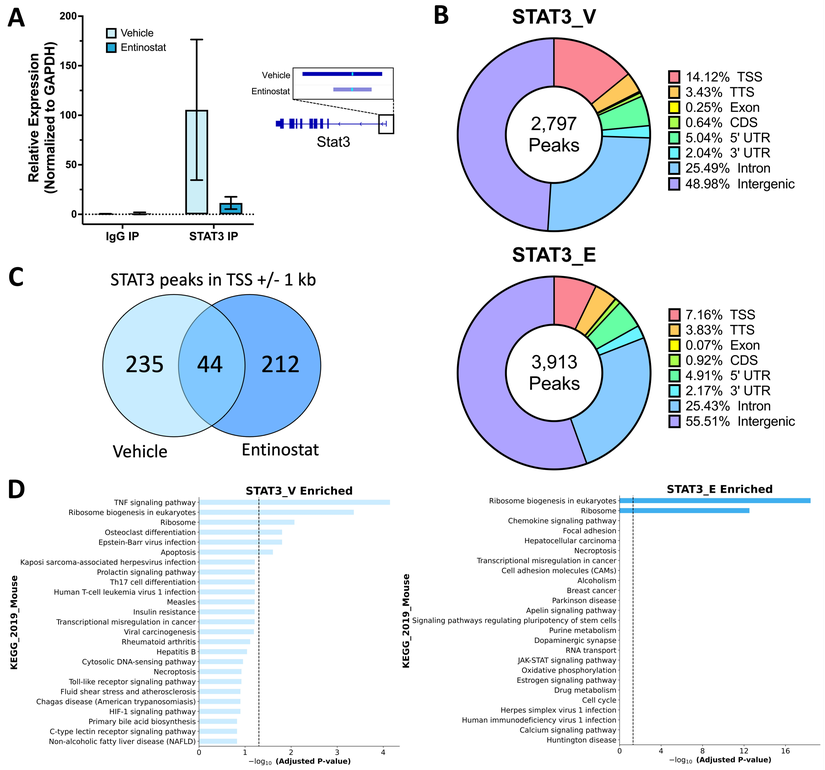


Fig. 1: HDAC inhibition decreases pSTAT3 in MDSCs from mice. A. Quantified western blots of isolated MDSCs from breast-to-lung metastases in 5 NT2.5-LM mice treated with vehicle or Entinostat reveals decreased pSTAT3 with Entinostat treatment. Each column is one mouse. Quantifications are adjusted density normalized to beta-actin. **p<0.01. **B.** Entinostat decreases pSTAT3 in J774M, MDSC-like cells.

HDAC inhibition of MDSCs decreases STAT3 enrichment in TNF signaling and apoptosis pathways



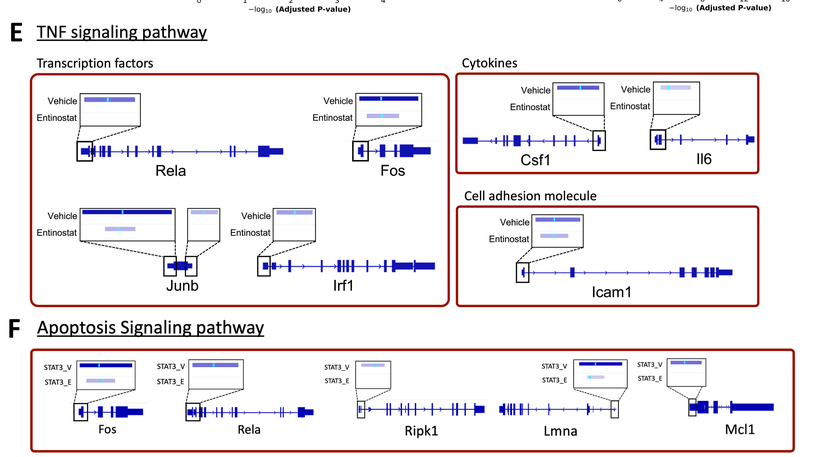


Fig. 2: HDAC inhibition decreases STAT3 enrichment at promoters of genes in TNF signaling and apoptosis pathways. A. ChIP-qPCR of IgG- vs. STAT3-IP genomic DNA at *Stat3* promoter, normalized to *Gapdh* promoter, showing positive enrichment that decreases with Entinostat. ChIP-seq NarrowPeak shows decreased 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 genes in the TNF signaling pathway and **F.** apoptosis pathway, including anti-apoptotic gene Mcl-1.

HDAC and STAT3 inhibition increases MDSC apoptosis

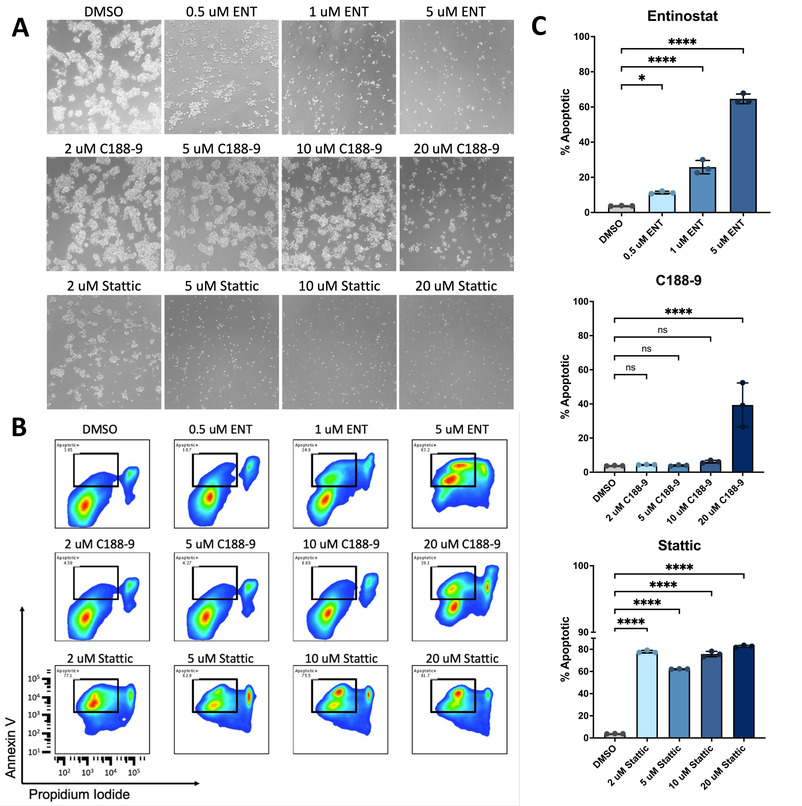


Fig. 3: HDAC and STAT3 inhibition increases MDSC apoptosis. A. Bright field images of J774M morphology after treatment with Entinostat and STAT3 inhibitors C188-9 and Stattic. **B-C.** Percentage of apoptotic (Annexin V+/Propidium Iodide-) J774M cells after Entinostat, C188-9, and Stattic treatment.

CONCLUSIONS

Our findings provide new evidence implicating a STAT3-mediated mechanism leading to decreased MDSC suppressive signaling and increased apoptosis 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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