Entinostat's modulation of myeloid derived suppressor cells through the STAT3-NFκB-AP-1 axis decreases suppressive signaling

Aaron G. Baugh[1], Edgar Gonzalez[1], Valerie H. Narumi[1], Sofi Castanon[1], Jesse Kreger[2], James Leatherman[3], Won Jin Ho[3], Ashley Cimino-Mathews[3], Vered Stearns[3], Roisin M. Connolly[4], Elizabeth M. Jaffee[3], 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 [3] Johns Hopkins University, Baltimore, MD [4] University College Cork, Cork, Ireland

Keck Medicine of USC

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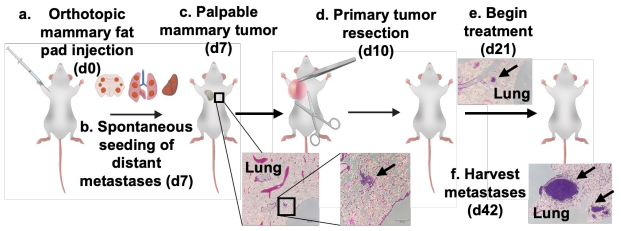
INTRODUCTION

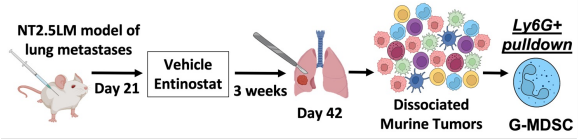
Metastatic breast cancer remains one of the leading causes of global cancer incidence in women, despite the benefit of immune checkpoint inhibitors (ICIs) in managing various cancers and improving patient quality of life. Metastatic breast cancer is characterized by extensive infiltration of the tumor microenvironment (TME) with immunosuppressive cells, such as myeloid derived suppressor cells (MDSCs), that inhibit anti-tumoral immune cells and prevent effective activation of the adaptive immune system by ICIs. Previously, our group has shown in the breast TME that epigenetic reprogramming of MDSCs by entinostat, a histone deacetylase inhibitor, decreases MDSC immunosuppressive function and contributes to response to ICIs, in part mediated by altered signaling within the Signal transducer and activator of transcription 3 (STAT3) and Nuclear factor kappa B (NFκB) axis[1,2]. Here, we sought to examine the effects of entinostat on MDSC reprogramming in a distant metastatic TME, with a focus on the STAT3-NFκB axis.

METHODS

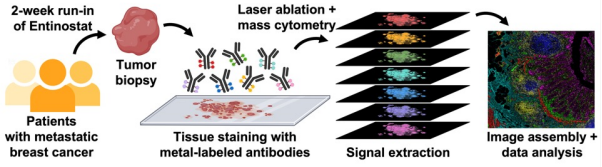
Using the syngeneic NT2.5LM NeuN mouse model of metastatic breast cancer, we established spontaneous lung metastases and treated with either vehicle or entinostat (5 mg/kg by oral gavage, 5x /week) for 3 weeks. Single cell RNA sequencing (scRNAseq) was performed on macro-dissected lung metastases.



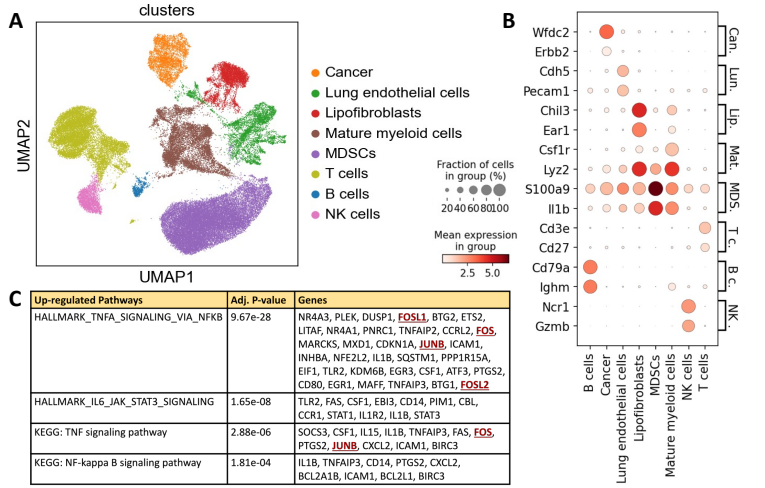
Using J774M cells, an MDSC-like cell line, and bead-isolated intratumoral granulocytic-MDSCs (G-MDSCs) from lung metastases, we performed western blot to probe for protein expression levels.



Using tumor biopsies from selected patients with metastatic breast cancer treated with entinostat (5 mg/week, 2-week run-in) during the clinical trial NCI-9844[3], we performed imaging mass cytometry (IMC) to identify and characterize immune cell populations.



RESULTS



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Up-regulated Pathways	Adj. P-value	Genes
HALLMARK_TNFA_SIGNALING_VIA_NFKB	9.67e-28	NR4A3, PLEK, DUSP1, FOSL1, BTG2, ETS2, LITAF, NR4A1, PNRC1, TNFAIP2, CCRL2, FOS, MARCKS, MXD1, CDKN1A, JUNB, ICAM1, INHBA, NFE2L2, IL1B, SQSTM1, PPP1R15A, EIF1, TLR2, KDM6B, EGR3, CSF1, ATF3, PTGS2, CD80, EGR1, MAFF, TNFAIP3, BTG1, FOSL2
HALLMARK_IL6_JAK_STAT3_SIGNALING	1.65e-08	TLR2, FAS, CSF1, EBI3, CD14, PIM1, CBL, CCR1, STAT1, IL1R2, IL1B, STAT3
KEGG: TNF signaling pathway	2.88e-06	SOCS3, CSF1, IL15, IL1B, TNFAIP3, FAS, FOS, PTGS2, JUNB, CXCL2, ICAM1, BIRC3
KEGG: NF-kappa B signaling pathway	1.81e-04	IL1B, TNFAIP3, CD14, PTGS2, CXCL2, BCL2A1B, ICAM1, BCL2L1, BIRC3

Fig. 1: ScRNAseq shows up-regulation of STAT3 and NFκB pathways in MDSCs upon entinostat treatment. 24 sets of NT2.5LM macro-dissected breast-to-lung metastases were sequenced using 10x Genomics Chromium Single Cell and processed using CellRanger v4.0.0. **A.** UMAP of identified cells using Louvain clustering, resolution parameter of 0.1, shows a large MDSC population (purple). Cell type identities were assigned using curated gene lists from previous work[1,2] and literature. **B.** Dotplot shows two representative gene markers for each cell cluster. **C.** Unsupervised pathway analysis of the MDSC cluster reveals significantly up-regulated STAT3 and NFκB signaling pathways with entinostat treatment. Transcription factors from the Activator protein 1 (AP-1) family in red.

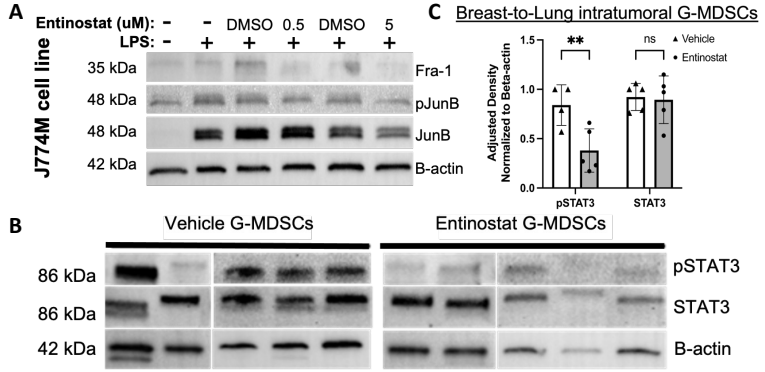
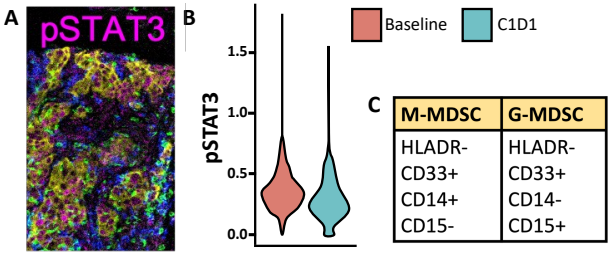


Fig. 2: Western blots of MDSCs show decreased expression/phosphorylation of transcription factors in the STAT3-NfκB-AP-1 axis upon entinostat treatment. A. Western blots of J774M, an MDSC-like cell line, treated with entinostat overnight and stimulated with LPS (1 ug/mL) for 2 hours reveal decreased AP-1 subunit Fra-1 expression, JunB expression, and JunB phosphorylation (pJunB). DMSO is vehicle control. **B.** Western blots of isolated G-MDSCs from breast-to-lung metastases in 5 mice treated with vehicle or entinostat (5 mg/kg) reveal decreased STAT3 phosphorylation (pSTAT3) with entinostat treatment. Each column is one mouse. **C.** Quantification of western blots in B., with adjusted density normalized to beta-actin. **p<0.01.



M-MDSC	G-MDSC
HLADR-	HLADR-
CD33+	CD33+
CD14+	CD14-
CD15-	CD15+

Fig. 3: IMC of tumor biopsies show decreased pSTAT3 in MDSCs in patients treated with entinostat. A. Representative IMC image of a tumor biopsy from a triple negative breast cancer patient responder from NCI-9844. Phosphorylated STAT3 (pSTAT3) is shown in pink. **B.** pSTAT3 is quantified at two timepoints: baseline and 2-week entinostat run-in (C1D1). IMC reveals a decrease in pSTAT3 in MDSCs upon entinostat treatment. **C.** IMC target antibody list that identifies the monocytic-MDSC (M-MDSC) and granulocytic-MDSC (G-MDSC) cells.

CONCLUSIONS

Crosstalk among the STAT3, NFκB, and AP-1 signaling pathways has been reported to regulate inflammation in breast cancer[4]. Our findings provide new evidence in the breast-to-lung metastatic tumor microenvironment (TME) that implicates a STAT3-NFκB-AP-1 mediated mechanism leading to decreased MDSC suppressive signaling upon entinostat treatment. Evaluating this mechanism in MDSCs taken directly from treated lung metastases represents one of the most biologically relevant mechanistic studies to date, and preliminary translation of findings in patients suggests that planned studies will lead to identification of a mechanism driving response to combination therapy of entinostat + ICIs.

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REFERENCES

1. Christmas, B. J., Rafie, C. I., Hopkins, A. C., Scott, B. A., Ma, H. S., Cruz, K. A., Woolman, S., Armstrong, T. D., Connolly, R. M., Azad, N. A., Jaffee, E. M., & Roussos Torres, E. T. (2018). Entinostat Converts Immune-Resistant Breast and Pancreatic Cancers into Checkpoint-Responsive Tumors by Reprogramming Tumor-Infiltrating MDSCs. *Cancer Immunology Research*, *6*(12), 1561–1577. https://doi.org/10.1158/2326-6066.CIR-18-0070
2. Sidiropoulos, D. N., Rafie, C. I., Jang, J. K., Castanon, S., Baugh, A. G., Gonzalez, E., Christmas, B. J., Narumi, V. H., Davis-Marcisak, E. F., Sharma, G., Bigelow, E., Vaghasia, A., Gupta, A., Skaist, A., Considine, M., Wheelan, S. J., Ganesan, S. K., Yu, M., Yegnasubramanian, S., … Roussos Torres, E. T. (2022). Entinostat decreases immune suppression to promote anti-tumor responses in a HER2+ breast tumor microenvironment. *Cancer Immunology Research*, canimm.0170.2021. https://doi.org/10.1158/2326-6066.CIR-21-0170
3. Roussos Torres, E. T., Rafie, C., Wang, C., Lim, D., Brufsky, A., LoRusso, P., Eder, J. P., Chung, V., Downs, M., Geare, M., Piekarz, R., Streicher, H., Anforth, L., Rudek, M. A., Zhu, Q., Besharati, S., Cimino-Mathews, A., Anders, R. A., Stearns, V., … Connolly, R. M. (2021). Phase I Study of Entinostat and Nivolumab with or without Ipilimumab in Advanced Solid Tumors (ETCTN-9844). *Clinical Cancer Research*, *27*(21), 5828–5837. https://doi.org/10.1158/1078-0432.CCR-20-5017
4. Ji, Z., He, L., Regev, A., & Struhl, K. (2019). Inflammatory regulatory network mediated by the joint action of NF-kB, STAT3, and AP-1 factors is involved in many human cancers. *Proceedings of the National Academy of Sciences*, *116*(19), 9453–9462. https://doi.org/10.1073/pnas.1821068116