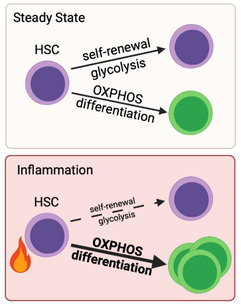
Itaconate-mediated regulation of hematopoietic stem and progenitor cell fate

University of Colorado Anschutz Medical Campus

Katia E. Niño[1], Taylor S. Mills[1,4], Zhonghe Ke[1], Rachel Culp-Hill[2], Angelo D'Alessandro[1,2], Craig Forester[3], Eric M. Pietras[1,4]

[1]Division of Hematology, Department of Medicine, [2]Department of Biochemistry and Molecular genetics,
[3]Division of Pediatric Hematology/Oncology/Bone Marrow Transplant[4] Department of Microbiology and Immunology,
University of Colorado Anschutz Medical Campus, Aurora, CO, USA

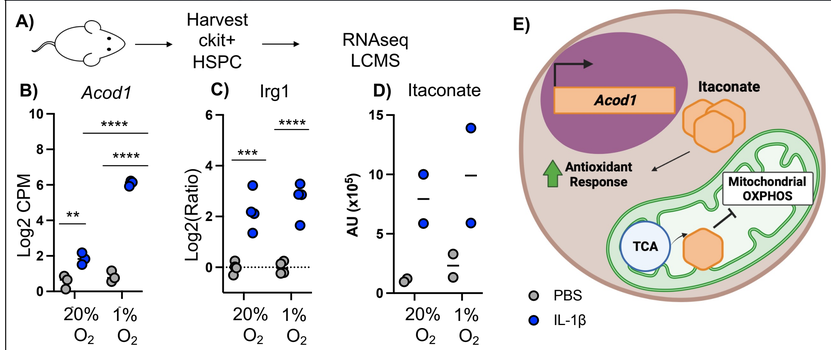
Introduction



- Hematopoietic stem and progenitor cells (HSPC) replenish all blood cell lineages over an organism's lifetime
- Inflammation increases mitochondrial OXPHOS in HSPC, promoting differentiation
- However, chronic inflammation does not lethally deplete HSPC

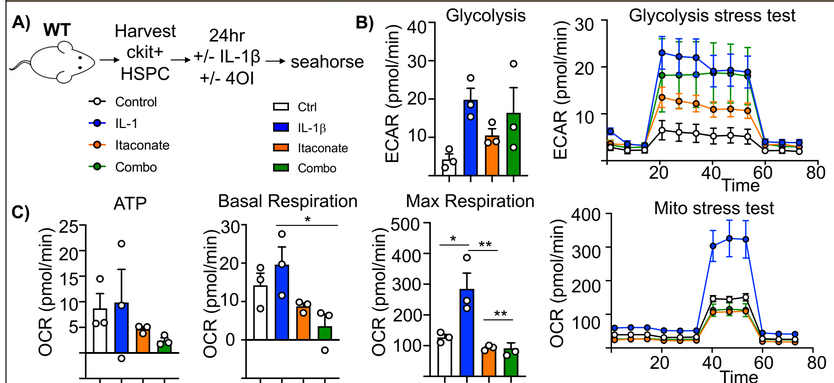
We *hypothesize* that HSPC elicit a secondary metabolic program that breaks inflammation-driven OXPHOS to prevent differentiation and subsequent exhaustion

IL-1β hyperinduces itaconate production in HSPC



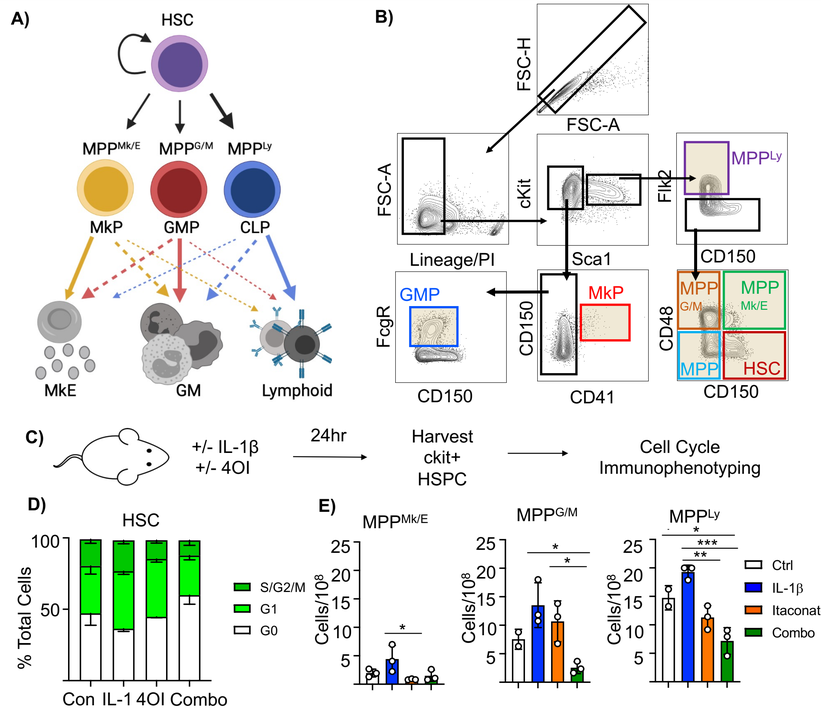
A) Experimental design for RNAseq and LCMS of WT cKit enriched HSPC cultured for 12hr +/- IL-1β **B)** *Acod1* transcript levels **C)** Irg1 protein levels from LCMS **D)** Itaconate levels from LCMS (*p<0.0332, **p<0.0021, ***p<0.002, ****p<0.0001 by one-way ANOVA) **E)** Cellular mechanism of itaconate

Itaconate limits IL-1β-mediated OXPHOS induction in HSPC



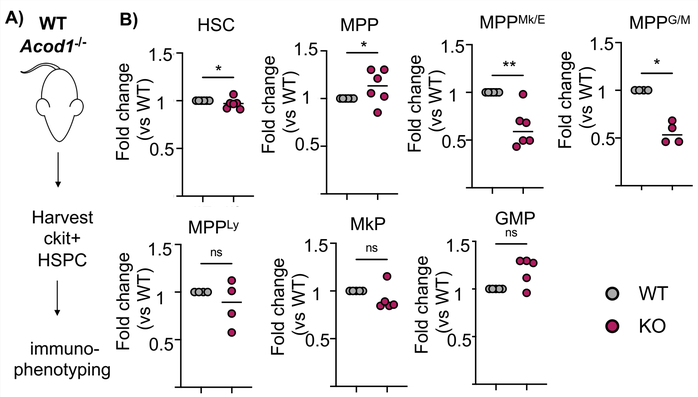
A) Experimental design for seahorse analysis of HSPC treated +/- IL-1β, +/- 4OI for 24 hr **B)** Glycolysis stress test of HSPC treated 24hr +/- IL-1β, +/- 4OI **C)** Mito stress test of HSPC treated 24hr +/- IL-1β, +/- 4OI (*p<0.0332, **p<0.0021 by one-way ANOVA)

Itaconate limits IL-1β-mediated HSC cycle entry and HSPC expansion



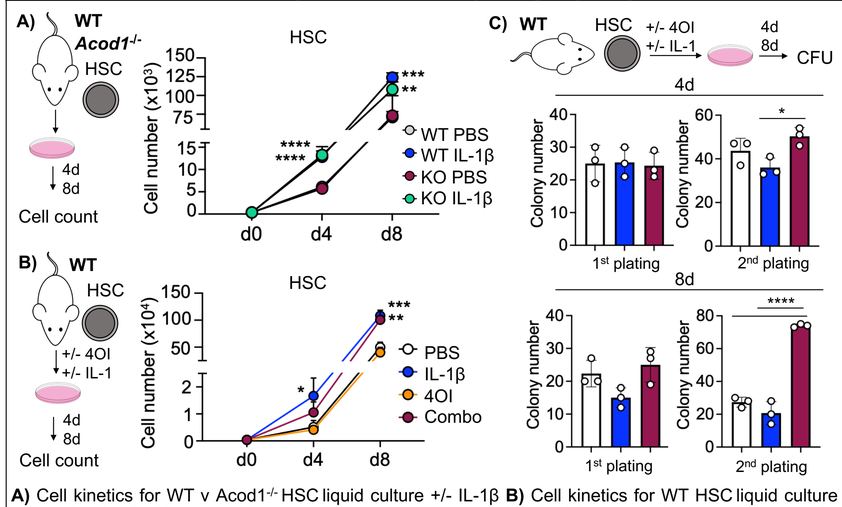
A) Hematopoietic tree **B)** HSPC gating strategy - ckit+ HSPC shaded in **C)** Experimental design for FACS immunotyping and cell cycle analysis of mice treated +/- IL-1β, +/- 4OI for 24 hr before HSPC harvest **D)** Cell cycle analysis of HSC from mice treated 24hr +/- IL-1β, +/- 4OI **E)** HSPC frequencies of mice treated 24hr +/- IL-1β, +/- 4OI (*p<0.0332, **p<0.0021, ***p<0.002, ****p<0.0001 by one-way ANOVA)

Acod1$^{-/-}$ mice have decreased bone marrow HSPC



A) Experimental design for FACS immunotyping HSPC from WT and *Acod1*$^{-/-}$ mice **B)** Fold change of HSPC frequencies of WT and *Acod1*$^{-/-}$ mice (*p<0.0332 by Mann-Whitney U-Test)

Itaconate promotes HSC replating capacity

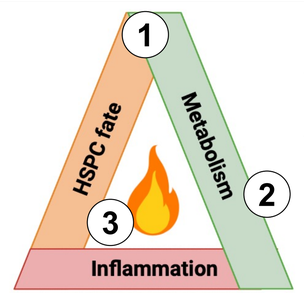


A) Cell kinetics for WT v Acod1$^{-/-}$ HSC liquid culture +/- IL-1β **B)** Cell kinetics for WT HSC liquid culture +/- IL-1β, +/- 4OI **C)** Methylcellulose colony formation assay of WT HSC cultured for 4/8 days +/- IL-1β , +/- 4OI (*p<0.0332, **p<0.0021, ***p<0.002, ****p<0.0001 by one-way ANOVA)

Final Conclusions

- Inflammation mediates the production of itaconate in HSPC
- Itaconate limits inflammation-mediated HSPC proliferation and differentiation
- Itaconate rescues HSC replating capacity

Future Directions



1) How do HSPC modulate metabolism through differentiation?

2) What is the molecular mechanism of itaconate in limiting HSPC proliferation/differentiation?

3) How do other hyperinflammatory conditions (e.g. leukemia, lupus, chemotherapy) maintain HSPC?

Acknowledgements

This work is funded by the CU Department of Medicine Outstanding Early Career Scholar Program, Cleo Meador and George Ryland Scott Endowed Chair in Hematology Research, R01DK119394, R01DK119394 Diversity Supplement, NSF GRFP (awarded to TSM)