**DIFFERENTIAL DEPENDENCE ON SPHINGOLIPID METABOLISM IN THE NORMAL AND LEUKEMIC HUMAN HEMATOPOIETIC HIERARCHY**

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**Introductio**n: Metabolic alterations are a hallmark of cancer but the lipid requirements of hematopoietic stem cells (HSC) in general, and leukemic stem cells (LSC) specifically are poorly understood. Sphingosine-1-phosphate (S1P) regulates HSC egress and lymphocyte trafficking. However, the intrinsic role of sphingolipid metabolism in the hematopoietic hierarchy or whether sphingolipid metabolism in LSC differs from HSC is unknown.

**Method**: By analyzing a comprehensive transcriptional roadmap of human hematopoiesis, and comparing this to a LSC signature developed from 84 human acute myeloid leukemia (AML) samples, we defined a lipid stem signature including *de novo* synthesis sphingolipid genes, whose expression is higher in HSC than progenitors. Unbiased clustering of RNA-seq data of 46 sphingolipid genes was sufficient to segregate mature erythroid, lymphoid, and myeloid cells suggesting a role in cell fate. To determine functionality of sphingolipid biology in normal hematopoiesis and human AML, xenograft assays, in vitro culture assays, lipidomics and proteomics were performed.

**Results**: Our lipid stem signature is enriched in LSC gene expression profiles by GSEA analysis, while S1P genes are enriched in non-LSCs suggesting that LSC and non-LSC have a differential dependence on sphingolipid metabolism as in normal hematopoiesis. Myeloid cells have the highest transcriptional expression of S1P receptors implicating the importance of S1P signaling in myeloid fate. Moreover, S1PR3 protein expression appears to be myeloid-specific. S1PR3 is highly overexpressed in AML relative to normal blood cells, suggesting S1P biology is dysregulated in AML. To assess a functional role in AML biology, we altered sphingolipid biology with inhibitors, including myriocin, which blocks de novo sphingolipid synthesis, and FTY720, a S1P mimetic, in mice bearing primary AML xenografts. Remarkably, myriocin and FTY720 both decreased leukemia burden in mice engrafted with primary AML, but only FTY720 treatment disrupted LSC function.

**Conclusions**: The normal human hematopoietic hierarchy displays a differential dependence on sphingolipid metabolism that is also distinct between HSC and LSC. Moreover, S1P receptor upregulation in AML over normal blood cells opens up a therapeutic window.

**Outcome/Impact:** Thus, targeting of bioactive sphingolipids is a viable novel therapeutic strategy in AML to eradicate LSC while sparing HSC.

**Keywords:** hematopoietic stem cells, leukemic stem cells, sphingolipid metabolism, acute myeloid leukemia