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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

NUP98-Rearranged Acute Myeloid Leukemia Stem Cells Superimpose Type I Interferon Signaling with Canonical Self-Renewal Programs

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The clinical outcomes of pediatric acute myeloid leukemia (AML) have shown improvement with combination therapies; however, relapse remains a significant concern due to the resistance of leukemia cells to conventional treatments. In some cases, epigenetic heterogeneity within leukemias can contribute to therapy resistance. For example, leukemia stem cells (LSCs) have been shown maintain AML, both before and after therapy. This study aims to identify LSCs within a mouse model of high-risk, *NUP98*-rearranged pediatric AML, and to identify mechanisms that sustain this population.

To identify and isolate a LSC subpopulation, we characterized murine *NUP98-HOXD13/Flt3-ITD*-driven AML in mice by performing cellular indexing of transcriptomes and epitopes (CITE-seq) analysis at two stages: pre-AML and fully transformed AML. Uniform Manifold Approximation and Projection (UMAP) revealed a highly reproducible pattern of heterogeneity within each AML specimen. Each AML had a small percentage of cells that resembled normal and pre-leukemic multipotent progenitors (MPPs), as well as additional populations with B-cell or myeloid gene expression. Pseudotime analysis indicated a trajectory from MPPs to mature cell-like lineages, implicating the MPP-like population as a potential LSC. This population expressed type I interferon (IFN-1) target genes more highly than non-LSC AML cells and pre-AML cells. This finding is significant because we previously identified IFN-1 as an effector of *Flt3-ITD/NUP98-HOXD13* synergy during AML initiation, and in human *NUP98*-rearranged AML (PMID: 36395068).

Differential gene expression analysis also identified several established self-renewal genes, including *Hlf*, *Myct1*, *Mpl*, *Slc7a8*, and *Gata2*, as highly expressed in putative LSCs relative to non-LSCs (figure B). This suggests that AML LSCs superimpose inflammatory signaling programs, particularly IFN-1, and core stem cell self-renewal programs to maintain a malignant state. The data raise the question of how IFN-1 shapes LSC identity.

We next sought to isolate and validate the putative LSC population. CITE-seq feature barcode data indicated high expression of CD117 and CD48, medium expression of B220, and low expression of Gr1 and CD11B in putative LSCs. We isolated LSCs based on these markers (Gr1-CD11b-B220 ^{Mid}CD117 ^{high}CD48+). Several non-LSC populations were isolated for comparison (e.g., Gr1+CD11B+ and Gr1-CD11B-B220 ^{high}CD117 ^{Mid} populations. Cytospin observation revealed no morphological differences among the three populations, but cell culture assays showed that only the Gr1-CD11b-B220 ^{Mid}CD117 ^{high}CD48+ cells exhibited proliferative capacity after multiple passages. Limit dilution transplant assays showed that the Gr1-CD11b-B220 ^{Mid}CD117 ^{high}CD48+ population was greatly enriched for LSC function relative to whole bone marrow (figure A).

Altogether, we have shown that we can prospectively isolate a LSC population from *NUP98-HOXD13/Flt3-ITD* AML. The LSC population is highly reproducible across specimens and it demonstrates high degrees of IFN-1 activity. Further work will establish the role of IFN-1/self-renewal gene interactions in LSC maintenance.

Disclosures No relevant conflicts of interest to declare.

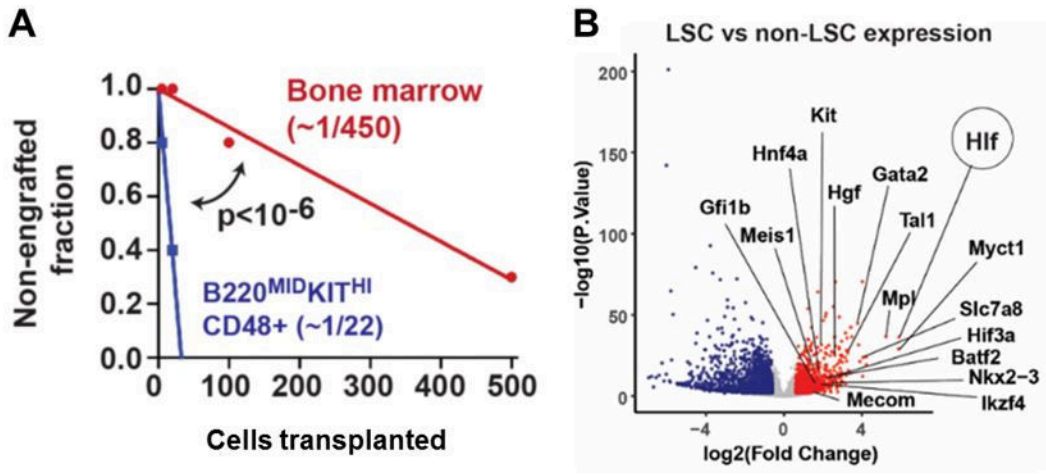

Figure 1. Self-renewal evaluation and differential gene expression in the putative LSCs. (A) Limit dilution transplants showing enrichment of LSC activity in the Gr1-CD11b-CD117^{High}B220^{Mid}CD48+ population. (B) Volcano plot showing differential gene expression in LSCs versus non-LSCs.

Figure 1

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