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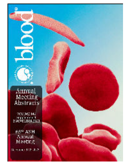

The 65th ASH Annual Meeting Abstracts

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602.MYELOID ONCOGENESIS: BASIC

Single-Cell RNA-Seq Reveals Intermediate Cell States and Identifies Features Defining Cellular Heterogeneity in Inv(16) Acute Myeloid Leukemia (AML)

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AML is an aggressive hematological malignancy with heterogeneous genetic abnormalities. One recurrent chromosomal translocation common in AML is chromosome 16 inversion, inv(16)(p13.1q22), which results in the leukemogenic fusion gene *CBFB- MYH11*. Using a conditional *Cbfb-MYH11* (CM) knock-in mouse model to mimic the somatic expression of the CM fusion gene, we demonstrated that CM expression leads to impaired hematopoietic differentiation as well as accumulation of phenotypic hematopoietic stem cells (HSC) and pre-megakaryocyte/erythrocyte (Pre-Meg/E) progenitors, which are predisposed to leukemic transformation. Flow cytometry-based phenotypic analysis revealed that the transformed leukemic blasts are predominantly characterized as pre-granulocyte-macrophage progenitor (pre-GM) (Lin $^{-}$cKit $^{+}$Sca1 $^{-}$CD16/32 $^{-/lo}$CD150 $^{-}$CD105 $^{-}$). However, cell type characterization via conventional surface markers is limited by known cell markers and is incapable of resolving pathologic heterogeneities. We previously reported that modeling AML in a continuum of differentiation states using single-cell RNA-seq (scRNA-seq) data predicts intermediate states of differentiation in AML. Herein, we utilized scRNA-seq to characterize CM leukemic blasts, and identified transcriptomic alternations and features that defined the leukemic cell states and heterogeneities. CM expression was induced by poly (I:C) and mice (n=3) were monitored for leukemia progression. At the leukemic end-stage (detection of >50% of circulating cKit $^{+}$ cells), we collected unfractionated and ckit $^{+}$ sorted peripheral blood mononuclear cells (PBMC) and bone marrow (BM) cells. Similarly poly(I:C)More than 105,000 cells harvested from the WT and CM leukemic mice were analyzed by scRNA-seq. We applied uniform manifold approximation and projection (UMAP) for dimensionality reduction and performed cell type annotation by SingleR. First, we mapped mature hematopoietic cell types in the WT PB and hematopoietic stem/progenitor cell populations (HSPC) in the WT BM, and in contrast, leukemic PB and BM were highly similar and showed distinct cell clusters compared to WT.

To characterize HSPC subpopulations, we focused on cKit+ sorted WT and leukemic BM cells, visualized them via UMAP, and performed clustering and cell type annotation. The leukemic cKit+ cells were mostly identified as megakaryocytic-erythroid progenitor (MEP)-like HSC, revealing a discrepancy between flow cytometry-based phenotypic characterization and transcriptome-based cell type assignment. These leukemic MEP-like HSCs showed up-regulation of erythroid differentiation genes, including *Hba-a1, Hba-a2, Hbb-bs, Hbb-bt* and *Sox6*, together with decreased expression of granulocytic markers such as *Elane, Prtn3, S100a8, S100a9* and *Mpo*. Comparison of leukemic MEP-like HSCs vs WT MEP clusters via gene set enrichment analysis (GSEA) with a threshold of nominal p-value $\leq$ 0.05 and FDR $\leq$ 0.05 in 50 hallmark gene sets (MSigDB) indicated dysregulated pathways, including up-regulation of "TNFα signaling pathway via NFκB", "inflammatory response", and "UV responsive downregulated genes"; and down-regulation of "MYC target V1", "MYC target V2", "E2F targets", "G2M checkpoint", and "oxidative phosphorylation". Similarly, GSEA in Gene Ontology (GO) showed downregulation of pathways relevant to mitochondrial protein translation and ribosomal biogenesis/assembly in leukemic MEP-like HSCs, compared to WT MEP. In addition, cKit+ BM cells can be separated into 31 clusters (C0-C30), of which 9 leukemic clusters were identified. Through gene expression-based cell cycle scoring, single sample gene set enrichment (ssGSEA), and marker identification,

these 9 leukemic clusters can be defined by several features, including high heme-metabolism (C0, C1, and C2), high proliferation (C1, C8), S phase (C4, C10), low oxidative phosphorylation (C2, C5, C17), high *Cd9* (C10), high *Egfl7* (C7, C8). Altogether, these data reveal intermediate differentiation cell states in inv(16) leukemic cells which are highly heterogeneous populations with distinct cell cycle stages, metabolic activities, and marker expression.

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