Adam Genda, BS

Authors: Genda A, Lee Y, Myers CL, Narendra VK, Millman S, Lowe S, Sachs Z

Single cell transcriptional profiles reveal inflamed states of leukemia stem cells in TP53Mut AML

Acute myeloid leukemia with TP53 mutations (TP53Mut AML) is a rapidly fatal blood cancer subtype with a high risk of relapse and a survival rate of less than 10% over two years. TP53Mut leukemia stem cells (LSCs) are a cell type resistant to chemotherapy and are defined by their ability to recapitulate the disease, leading to relapse. Identification of therapeutic targets to target and block self-renewal would prevent disease progression and relapse in TP53Mut AML.

Prior work has demonstrated that TP53Mut AML has an inflamed microenvironment characterized by dysfunctional T cells. Previously, we found that mutant p53 protein directs the expression of inflammatory mediators including the NFkB/STAT signaling pathway in human AML cell lines and primary human AML samples. Furthermore, both mutant p53 and the NFkB/STAT pathway have been implicated in enhanced LSC function in AML. Therefore, we hypothesize that mutant p53 confers self-renewal in TP53Mut AML via the activation of NFkB-mediated inflammatory pathways. The transcriptional profiles of human AML have been widely published, but have not been reported for LSCs from TP53Mut human samples. Therefore, we performed single cell RNA sequencing of primary human AML samples and analyzed the single cell transcriptional profiles of TP53Mut and wild type AMLs of both primary and patient-derived xenograft samples.

We compared standard cell type annotation approaches and found SingleR to offer the most precise identification using established AML cell type reference profiles. Additionally, we applied our previously developed novel method for LSC identification, Single Cell Correlation Analysis to pinpoint critical subsets of LSCs. Next, we used CopyKAT-based copy number annotation to identify tumor subclones with distinct aneuploidy and confirmed these results with clinical karyotyping. Next, we used gene set enrichment analysis to identify shared expression pathways in LSCs versus nonLSCs across samples. Consistent with our earlier findings and published literature, we found that LSCs from TP53Mut AML samples display significant and consistent dysregulation of the NFkB-mediated

inflammatory pathways, which may be important for enhanced LSC function driven by mutant p53 proteins. Notably, these inflammatory pathways can be therapeutically targeted. Our data suggests that targeting these pathways may be an effective strategy to kill TP53Mut AML LSCs.