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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

IKZF2 Specifically Participates in AML1-ETO Mediated Blockage of Myeloid Differentiation

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Objectives: The chromosomal translocation (8;21) is one of the most recurrent cytogenetic aberrations in acute myeloid leukemia (AML). Although most patients achieve complete remission, one third still relapse due to the persistent leukemia stem cells (LSCs). The lack of significant improvements in outcomes, especially in elderly patients ineligible for standard chemotherapy, has spurred further investigation in t(8;21) leukemia. In this study, we investigate the mechanism of AML1-ETO (AE) fusion gene induced differentiation blockage from earlier stages of HSPCs, aiming to identify potential therapeutic targets that can effectively overcome treatment resistance and minimize long-term side effects.
Methods: The AML1-ETO conditional knock-in mouse (Aml1 $^{Eto/+}$; Mx1-Cre) was previously established, hereafter called AE ^{KI}. The Cas9-expressing AE ^{KI} model, generated by crossing AE ^{KI} and Cas9 mice strains, was employed to define the regulatory program of AE fusion gene. For knockout of AE and IKZF2, Kasumi-1 cells were transduced with the single-vector lentiviral system containing sgRNAs for the respective genes, or a nontargeting control. Colony formation and serial bone marrow transplantation assay were performed to evaluate the function of HSPCs. ATAC sequencing, transcriptional profiling (RNA-seq) and Cut&Tag for H3K4me3, H3K27me3, H3K27ac were performed and analyzed comprehensively to characterize the regulatory profile of AE fusion gene.
Results: Our previous research demonstrated that induction expression of AE fusion gene in vivo resulted in excessive accumulation of Lin $^{-}$Sca-1 $^{+}$c-Kit $^{+}$ (LSK) cells, leading to differentiation blockage from HSCs to HPCs. IKZF2 showed significantly higher expression in LSK cells following PIPC induction of AE expression compared to that of the control group. IKZF2 was highly increased in AML patients harboring AE fusion gene. And the level of IKZF2 was significantly higher in human t(8;21) cell lines Kasumi-1 and SKNO-1 when compared to that of non-AE controls. Depletion of the AE fusion gene led to a dramatic decrease in IKZF2 expression, while constitutive expression of the AE fusion protein in U-937 cells resulted in elevated levels of IKZF2. Loss of IKZF2 resulted in apoptosis in AE-expressing leukemia cells, and led to an increase in the myeloid differentiation markers CD11b and CD14. These findings suggested a functional relevance of IKZF2 in AE-driven AML.
In our Cas9-expressing AE ^{KI} model, a significant improvement in the peripheral chimerism rate was observed following IKZF2 knockout at various time points. The population of HSCs, mainly the ST-HSCs, was reduced, while the more differentiated MPP and Lin $^{-}$Sca-1 $^{-}$c-Kit $^{+}$ (LK) pools, especially the GMP and CMP groups, were expanded. These findings indicated that the differentiation block triggered by AE was partially relieved by IKZF2 knockout. Furthermore, the deletion of IKZF2 had minimal impact on normal myeloid differentiation, highlighting its specific role in AE settings.
We further investigated the underlying mechanisms through multi-omics analysis. The induction of AE led to increased accessibility of the myeloid differentiation inhibitory elements, including IKZF2. CUT&Tag data confirmed a significant decrease in H3K27me3 signals at the promoter regions of IKZF2 compared to that of the control group. Regarding the role of IKZF2 in chromatin remodeling, it is likely to interact with AE to form an epigenetic-transcriptional control module. This may expand the signal for differentiation blockage and drive critical leukemogenic gene expression profiles.

Conclusions: Our results demonstrate that IKZF2 is a target gene of AML1-ETO crucial for AML1-ETO-induced differentiation blockage from HSCs to HPCs. The epigenetic regulation mechanism may contribute to the AML1-ETO-IKZF2 regulatory axis. And targeting IKZF2 may become a potential therapeutic strategy in human t(8;21) AML especially for the eradication of AML1-ETO-associated LSCs.

Disclosures No relevant conflicts of interest to declare.

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