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IKZF2 Driving Self-Renewal and Inhibiting Myeloid Differentiation Through Chromatin Accessibility

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Abstract

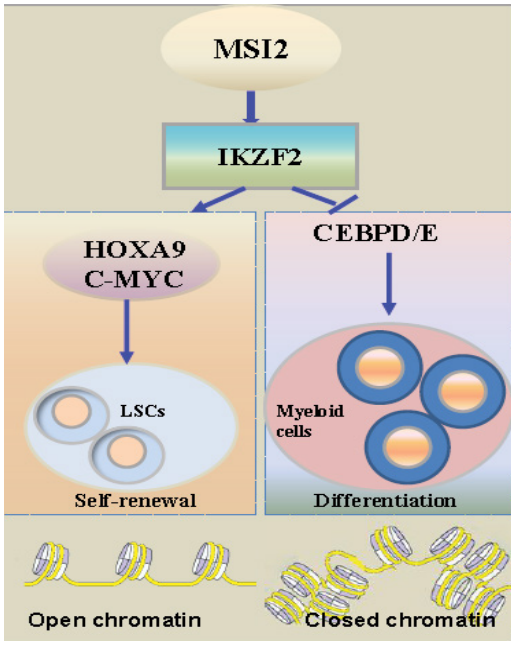
Control of myeloid differentiation and leukemia stem cell (LSC) self-renewal involves genetic and epigenetic regulators and mechanisms. Genetic regulation in leukemia has been highlighted as a novel way for maintaining the LSC program. Translocations of histone methyltransferases, such as the mixed-lineage leukemia (MLL) genes including *Hoxa9*, *Myc*, and *Ikzf2*, give rise to one of the most aggressive subtypes of acute myeloid leukemia (AML). Uncontrolled expansion of immature myeloid cells coupled with leukemia stem cell self-renewal and a block in differentiation are some of the characteristic traits of AML.

Keywords: Leukemia; Genetic and epigenetic regulators; Cellular metabolism

Introduction

In the last few years, there is increasing interest in uncovering the role of genetic and epigenetic regulators in the AML progression, but analysis of this mechanism in leukemia stem cells is still only in its infancy. Like normal hematopoietic stem cells, LSCs are thought to be capable of self-renewal during which they give rise to an identical copy of themselves as well as to a differentiated daughter cell by asymmetrical division [1-3]. Those stem cells have the capacity to be serially transplanted and can give rise to differentiated cells, recreating the complete phenotypic heterogeneity of primary leukemia. Failure to eradicate LSCs with minimal residual disease can lead to relapse. Consequently, AML subtypes with higher frequencies of LSC are associated with poorer outcome [4]. Interestingly, certain genes enriched in LSCs are shared with normal HSCs constituting a stemness program that is an independent predictor of patient overall survival. Thus, pathways that maintain LSC self-renewal, enhance LSC survival, or suppress LSC differentiation represent important therapeutic opportunities for the treatment of AML.

In the recent study, Park and his colleagues demonstrated that deletion of *Msi2* from hematopoietic compartment results in delayed leukemogenesis, reduced disease burden, and a loss of LSC function in a murine leukemia model [2]. Transcriptional profiling of these *Msi2*-deficient animals revealed a loss of the hematopoietic/leukemic stem cell self-renewal program and an increase in the differentiation program. In this issue, Park et al. focused on *Ikzf2*, a member of the Ikaros transcription factor family. *Ikzf2* has been shown to be the most-downregulated gene in *Msi2*-deficient LSCs, which have a self-renewal defect. Depletion of IKZF2 in bone marrow leukemic cells resulted in reduced proliferation, increased differentiation and increased apoptosis. In line with these results, genomic profiling and chromatin accessibility analysis demonstrated that IKZF2 loss led to increased myeloid differentiation. This phenotypic change correlated with the increased chromatin accessibility of transcription factor binding sites for C/EBPδ and C/EBPε, which are important for cytokinin-induced granulocytic differentiation in leukemic cells and terminal differentiation of cells in the myeloid lineage, respectively (Figure 1). In contrast to the increased myeloid C/EBP program, acute deletion of *Ikzf2* induced the reduction of accessibility for HOXA9 and c-MYC binding sites, suggesting that it could be a direct effect of IKZF2 loss rather than a



Note: MSI2 mediates self renewal and differentiation program by interacting and retaining efficient translation of IKZF2. IKZF2 is regulated by MSI2 and maintains open accessibility in self-renewal transcription factor motifs such as at the HOXA9 sites and C-MYC sites whereas motifs of differentiation transcription factors including C/EBPs are kept closed.

Figure 1: The chromatin accessibility regulate stemness and differentiation in leukemic stem cells.

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downstream effect from the activation of the differentiation program. However, while *Ikzf2* has been shown to be highly expressed in LSCs as a chromatin remodeler in a murine MLL-AF9 model and its deficiency results in defective LSC function, it is required for leukemogenesis but dispensable for normal hematopoiesis. These data suggest that IKZF2 is differentially required in myeloid leukemia cells compared to normal cells [5].

Discussion

How do IKZF2 defects lead to a myeloid differentiation block in leukemia stem cells? As mentioned above, IKZF2 depletion does not seem to affect the normal hematopoietic cell survival and self–renewal, and it is dispensable for normal hematopoiesis. What is the link? Chromatin accessibility states between LSCs and HSCs contribute to the mechanism. Chromatin accessibility is differentially regulated across the genome, and the organization of accessible chromatin reflects a network of permissible physical interactions through which enhancers, promoters, insulators and chromatin-binding factors cooperatively regulate gene expression [6]. Chromatin accessibility plays a key role in regulating cell type specific gene expression during hematopoiesis but has also been suggested to be aberrantly regulated during leukemogenesis [7]. However, the role of regulatory elements in initiating accessibility remodelling and how these epigenetic features are dynamically established to control gene expression areneed to be further explored. Thus, the author found the regions with altered accessibility contained IKZF2 motifs, indicating that IKZF2 binding could directly affect chromatin accessibility. IKZF2 loss leads to reduced accessibility in intronic enhancers whereas it increased accessibility at promoters. Chromatin accessibility is influenced both by the density of associated proteins (particularly histones) along DNA and the fractional residence time of these factors [6]. Motif enrichment analysis from the combinatorial assessment of RNA-sequencing, chromatin accessibility by ATAC-seq and direct binding of IKZF2 by the cut-and-run assay in MLL-AF9 LSCs identified the C/EBPδ and C/EBPε as the most accessible motifs whereas HOXA9 motif became less accessible in the *Ikzf2* deleted LSCs. IKZF2 maintains open accessibility in self-renewal transcription factor motifs such as at the HOXA9 sites whereas motifs of differentiation transcription factors including C/EBPs are kept closed. Recent advances suggest that homeostatic maintenance of accessibility is itself dynamically regulated through a competitive interplay between chromatin-binding factors and nucleosomes [6]. Imposed expression of HOXA9 could partially differentially rescue stem cell proliferation, differentiation and apoptosis effects in *Ikzf2* deleted cells. In addition, C/EBPε depletion by shRNAs partially rescued the effects of IKZF2 depletion. Therefore, IKZF2 differentially maintains the accessibility and expression of the self-renewal and differentiation genes in LSCs (Figure 1). Employing the Cre-ER expressing MLL-AF9 LSCs, they validated that myeloid differentiation related gene *C3, Fpr2, S100a8* and *S100a9* were upregulated after acute deletion [8-10]. Next, there should be a significant therapeutic window for this approach. IKZF2 can act as a chromatin remodeler that regulates the *Hoxa9* driven self-renewal program and inhibit differentiation *C/ebp* gene expression program in LSCs, suggesting targetable differences in the regulation of quiescence between HSCs and LSCs. This finding may provide a rational role for Ikzf2 as a tumor suppressor in lymphoid malignancies and therapeutically target IKZF2 in myeloid leukemia. More evidence and future studies will be needed to dissect its role in human AML.

See related article:

Citation: Sun-Mi P, Cho H, Thornton AM, Barlowe TS, Chou T, et al. (2019) IKZF2 drives leukemia stem cell self-renewal and inhibits myeloid differentiation. Cell Stem Cell 24: 153 to 165.e7.

Conclusion

Despite the emerging evidence for the influence of chromatin accessibility in the leukemic stem cell differentiation and self-renewal progression including IKZF2 regulation of the accessibility of HOXA9 and CEBPδ/ε motifs, regulation of gene expression in leukemia stemness and response pathways have, on the whole, been still poorly defined. Importantly, the relationship between epigenetic regulators and leukemia progression may not be confined to normal stem cells. Further studies should address these questions. An attractive future perspective of this field of research is to discover new venues and new therapeutic targets to control the functions of LSCs by modulation of chromatin accessibility in LSCs. Clinical targeting still remain challenging and further studies are required to determine the general applicability of their findings across AML subtypes.

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Conflicts of Interest

No potential conflicts of interest were disclosed.

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