● ● ● **IMMUNOBIOLOGY**

Ikaros: the enhancer makes the difference

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In this issue of *Blood*, Yoshida et al present a detailed evaluation of the transcriptional regulation of *Ikzf1* (Ikaros) expression during hematopoiesis.[1]

Ikzf1 (Ikaros) functions as a master regulator of hematopoiesis, a modulator of immune responses, and a tumor suppressor in leukemia.[2,3] During hematopoiesis, Ikaros plays major roles during at least 2 different stages of differentiation: (1) at the level of the lymphoid-primed multipotent progenitor (LMPP), where the absence of Ikaros results in failed lymphoid differentiation[4,5]; and (2) at early stages of T- and B-cell differentiation—in double-positive thymocytes and in pre-B cells—where reduced Ikaros function is associated with malignant transformation in both mice and humans.[6-8] The importance of Ikaros for clinical hematology and oncology was underscored when *Ikzf1* haploinsufficiency was shown to result in acute lymphoblastic leukemia with a high risk for relapse.[9] Our knowledge of the regulatory network that controls *Ikzf1* transcription is very limited. Due to the requirement for fine-tuned control of Ikaros levels for normal hematopoiesis, it is logical for its transcription to be controlled by complex mechanisms that are specific for each stage of differentiation. Dissecting the mechanisms for transcriptional regulation of *Ikzf1* expression is thus of paramount importance for understanding hematopoiesis and malignant transformation and will profoundly impact clinical science.

Using a mouse transgenic reporter approach, combined with chromatin immunoprecipitation and next-generation sequencing (ChIP-SEQ), Yoshida et al[1] functionally evaluate the highly conserved enhancer regions of the *Ikzf1* locus. Previous studies by this group identified 10 putative regulatory regions at the *Ikzf1* locus.[10] The evolutionarily conserved putative *Ikzf1* enhancers were tested in vivo for their ability to promote transcription of a green fluorescent protein (GFP) reporter under the control of the main *Ikzf1* promoter. For each of these, a putative enhancer reporter cassette was produced and used to generate multiple transgenic mouse lines. GFP expression was evaluated in peripheral leukocytes and compared with GFP expression in a reporter cassette driven by the GFP promoter alone.

The *Ikzf1* enhancers were evaluated for their ability to (1) counteract transcriptionally repressive chromatin; (2) increase transcription levels in permissive chromatin; and (3) stimulate cell type–specific transcription
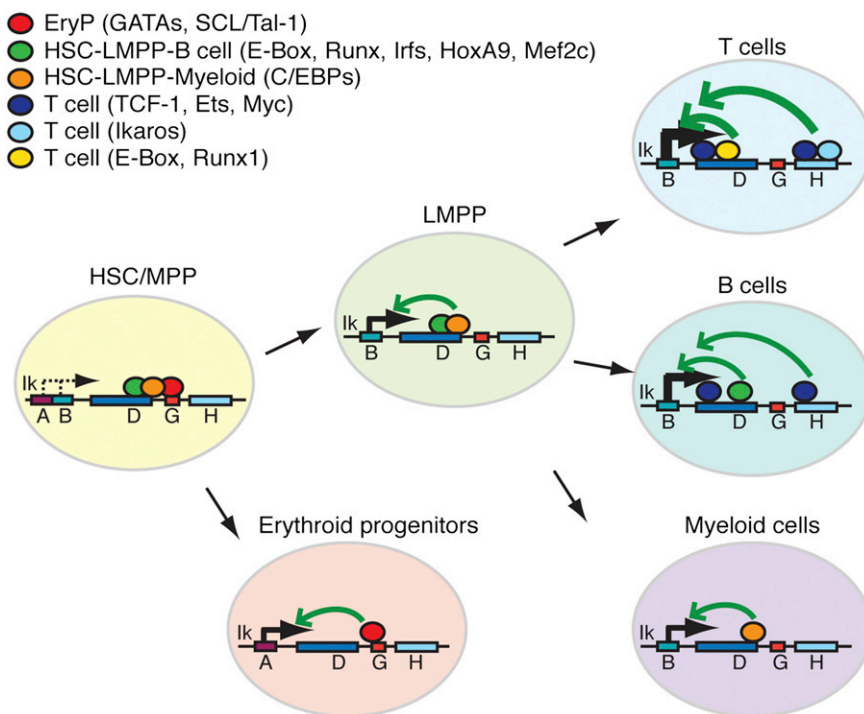
Ikzf1 enhancer-promoter interactions during hematopoiesis. Lineage and/or stage associated transcription factor binding at enhancers of the *Ikzf1* locus. Lineage-specific transcription factors at these sites are depicted as color-coded circles. Arrows indicate potential interactions between cell type–specific enhancers and promoters supporting *Ikzf1* expression at appropriate developmental stages.

during different stages of lymphoid and myeloid lineage differentiation. The results identified enhancers F, H, and I as potent stimulators of transcription, but only in permissive chromatin, thus acting similarly to classical enhancers that promote initiation and elongation of transcription. In contrast, enhancers D and J were efficient in counteracting repressive chromatin, which likely is the result of recruitment of epigenetic factors that promote a transcriptionally permissive chromatin state to that region.

All of the *Ikzf1* enhancers were able to stimulate the *Ikzf1* promoter-based expression in B and myeloid cells. However, only 2 enhancers (D and H) were able to stimulate transcription in T cells as well. The D enhancer was the only one capable of stimulating GFP expression above the basal level in the LMPP that marks the earliest stage of lymphoid differentiation. Further analysis revealed that enhancer D is critical in counteracting repressive chromatin at the *Ikzf1* locus and for maintaining a high level of transcription, and these functions could not be compensated by any other *Ikzf1* enhancers. The authors further dissected the enhancer D and identified subdomains that confer stage-specific expression in T-lineage cells.

Although individual enhancers were capable of stimulating *Ikzf1* expression, their activity could not replicate the activity of the wild-type endogenous *Ikzf1* locus. The endogenous gene expression pattern in hematopoietic cells and in the neuronal lineage was ensured when 9 of the 10 conserved *Ikzf1*enhancers were combined in a miniregulatory locus.

The authors conclude these studies by analyzing previous ChIP-SEQ data to identify a network of transcription factors that bind in vivo at the *Ikzf1* enhancers. These analyses revealed binding of HEB, runt-related transcription factor 1 (Runx1), T-cell factor 1, and Ikaros in thymocytes; avian myelocytomatosis oncogene (c-Myc) and avian erythroblastosis virus E26 oncogene 1 (Ets-1) in B cells; and GATA binding protein 1 (GATA1), GATA2, and stem cell leukemia/T cell acute lymphocytic leukemia 1 (SCL/Tal1) in erythroid precursors. In addition, motif search for transcription binding sites at enhancers D and H identified enrichment of binding sites for several transcriptional factors with important roles in hematopoiesis; eg, Runx, Homeobox

A9 (HoxA9), special AT-rich sequence binding protein 1 (Satb1), Interferon regulatory factor-1 (Irf1), Irf4, CCAAT/enhancer binding protein–α (C/EBP-α), C/EBP-β, myocyte enhancer factor 2C (MEF2C), and E2A. The proposed working model by Yoshida et al, based on these findings, is outlined (see figure).

What are some of the implications? Next-generation sequencing has identified inactivating deletions and mutations at the *Ikzf1* locus in a large subset of B-cell precursor acute lymphoblastic leukemia (B-ALL) and in early T-cell ALL in humans. These genetic alterations that result in reduced *Ikzf1* activity are poor prognostic indicators in pre-B-ALL. The identification of *Ikzf1* enhancers that are essential for optimal *Ikzf1* expression provides an additional tool to identify potential prognostic markers. The obvious next step would include sequencing *Ikzf1* enhancer elements and correlation of potential mutations and/or polymorphism in these regions with the development and/or outcome of leukemia. With the rapid development of next-generation sequencing technology and the decreased cost of sequencing, these assays are quite feasible and may yield important diagnostic information.

The identification of a network of transcription factors that positively regulates *Ikzf1* expression provides an opportunity to uncover larger signaling pathways that control normal and malignant hematopoiesis. Besides the obvious impact on scientific advances in the field, this could also have important therapeutic implications. The modulation

of signaling pathways that control *Ikzf1* expression could be a powerful tool for the treatment of hematopoietic malignancies and some immunological disorders. This story is just developing.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● **CLINICAL TRIALS & OBSERVATIONS**

Can CRd be a standard for refractory myeloma?

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In this issue of *Blood*, Wang et al describe that the combination of carfilzomib, lenalidomide, and low–dose dexamethasone (CRd) can be a valuable option in relapsed and refractory multiple myeloma patients.[1]

In 1958, Blokhin et al reported the first experience with sarcolysin (melphalan) in neoplastic diseases,[2] and a few years

later Drs D. Bergsagel and R. Alexanian pioneered studies showing the efficacy of melphalan in multiple myeloma. However,