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The Ikaros family in lymphocyte development

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The IKZF family of transcription factors are essential regulators of lymphopoiesis. Ikaros, Helios, Aiolos and Eos function as transcriptional repressors and activators during T and B cell differentiation and in mature cell function, depending on the stage of development and/or cell type. Their potential mechanisms of action are varied. Ikaros family proteins partner with multiple complexes, including NuRD, PRC2 and transcription elongation factors, to modulate gene expression and the chromatin state. In humans, mutations in the IKZF genes are associated with B cell deficiency, leukemias and autoimmunity. In this review, we focus on the function of Ikaros family proteins in early T and B lymphocyte development, and discuss the molecular and physiological activities of this family.

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Introduction

The Ikaros zinc finger transcription factor (encoded by the IKZF1 gene) was identified in the early 1990s by the Smale and Georgopoulos groups as a regulator of lymphocyte-specific genes [1–3]. Subsequently, four other homologous proteins were identified — Helios, Aiolos, Eos and Pegasus (encoded respectively by IKZF2-5) (Figure 1) (for review, see [4]). All contain a remarkably conserved N-terminal DNA-binding domain (DBD), comprising four C2H2 zinc fingers (ZFs), that binds sequences containing the core GGGAA motif (except Pegasus), and a C-terminal dimerization domain, comprising two additional C2H2 ZFs [5–7]. These proteins have ancient roots in animal evolution, as homologs have been found in the fly and worm. While non-hematopoietic functions have been ascribed, hematopoietic cells are the main functional targets of the Ikaros family. These factors exert pleiotropic functions in almost all hematopoietic cell types, from stem cells to mature lymphoid and myeloid cells.

The lymphoid system is especially affected by perturbations in Ikaros family function. Both recent and older data have highlighted the crucial roles of Ikaros proteins in murine T and B cell differentiation, and these findings have clear implications for human pathologies like leukemias or immunodeficiencies where the IKZF genes are mutated. Lymphoid cells have also been used as model systems to understand how Ikaros proteins work at the molecular level. Here we review recent advances in the physiological and molecular functions of this family during T and B cell development.

Genetics of IKZF1-4 in mouse and man

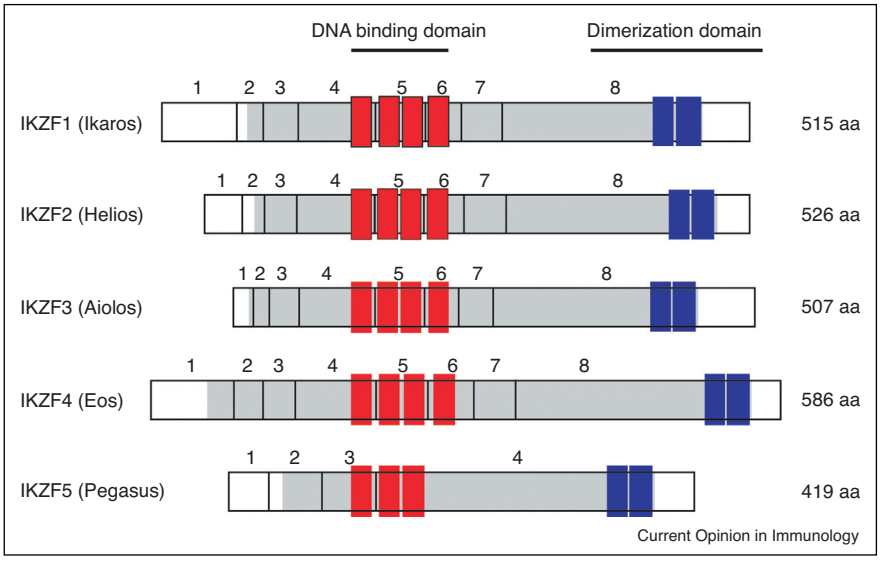
To understand their *in vivo* function, germline, lymphocyte-specific and inducible mouse models for Ikaros proteins have been generated (Table 1). For Ikaros, hypomorphic, null and dominant-negative (dn) alleles are available, while null mutations have been reported for Helios, Aiolos and Eos. These tools have revealed important functions for every member in T and B cell biology (see below).

In man, genetic lesions and polymorphisms have linked the IKZF1–4 genes to a myriad of diseases affecting lymphocyte differentiation, homeostasis or function. Haploinsufficient germline IKZF1 loss-of-function (LOF) mutations are associated with common variable B cell immunodeficiency (CVID), and with autoimmunity in the forms of systemic lupus erythematosus (SLE) and immune thrombocytopenic purpura [8••,9••]. Somatic IKZF1-3 LOF mutations are found in B cell precursor-acute lymphoblastic leukemia (BCP-ALL) and early T cell precursor (ETP)-ALL [10–12]. IKZF2 LOF mutations are also recurrent in adult mature T cell leukemias triggered by HTLV infection [13]. Further, genome-wide association studies (GWAS) have linked specific polymorphisms at or near the IKZF1-4 loci to increased susceptibility to autoimmune or inflammatory diseases, like SLE, Crohn's disease and asthma. These results have revealed striking similarities in Ikaros family function between mouse and man.

Ikaros proteins in T lymphocytes

In the mouse, fetal thymocyte development requires Ikaros, though T cell differentiation occurs in post-natal Ikaros null mice, and cKit+ early T lineage progenitors

Figure 1



Primary structure of IKZF1-5 transcripts and proteins. Schematic representation of the mRNA transcripts for the murine IKZF1-5 genes. The exons for each gene are numbered 1–8. The coding regions are in gray, the non-coding in white. The DBD ZFs are in red; the dimerization ZFs are in blue.

(ETPs) are detected in the thymus [14,15]. However, T cell development is aberrant. The biggest changes occur in αβ T cells, where pre-T cell receptor (pre-TCR) and TCR signaling are amplified in CD4−CD8− DN and CD4+CD8+ DP thymocytes, leading to enhanced maturation and increases in positive selection [16,17]. Ikaros affects gene expression at every stage of T cell differentiation, but particularly genes whose expression levels are sharply increased or decreased from one stage to the next. In Ikaros knockdown thymocytes, the expression of these stage-specific genes are deregulated [18], suggesting that Ikaros is required for dynamic gene expression changes during thymocyte development (Figure 2).

Inevitably, Ikaros deficiency leads to the early and aggressive development of T cell acute lymphoblastic lymphomas/leukemias in all mutant mice, regardless of mutation (for review, see [19]), suggesting that Ikaros is a powerful tumor suppressor in T cells. In thymocytes, Ikaros helps to shut down the Notch pathway in DN4 and DP cells [20,21], and Ikaros loss leads to an expansion of the DN4 compartment through increased Notch-dependent proliferation [20]. Indeed, Ikaros appears to shape the target gene response to Notch activation in terms of repertoire and timing after the β-selection checkpoint [21]. As Notch activation is required for early T cell differentiation but is oncogenic after the DP stage, Ikaros repression of the Notch pathway is the primary mechanism of its tumor suppressor function in murine T cells. Interestingly, Ikaros mutations are not associated with Notch activation in human T-ALL (see [19]). That is likely to be due to the differential way in which the Notch1 receptor is activated in murine versus human T cells. In mice, Notch1 is frequently activated through a non-conserved, RAG-mediated deletion of its exon1 and adjacent promoter sequences [22,23], and Ikaros deficiency cooperates by activating a cryptic intragenic promoter that produces transcripts encoding constitutively active Notch1 proteins [23,24].

Ikaros appears to be the dominant family member during thymocyte development. Aiolos null thymocytes and peripheral T cells do not display detectable deficiencies, even though Aiolos is highly expressed in T cells from the DP thymocyte stage on [25]. Helios and Eos are implicated as markers of regulatory T (Treg) cell subsets and in the maintenance of Treg cell function [26,27], but they have so far not been required for thymocyte differentiation [28].

Ikaros proteins in B lymphocytes

Similar to T cells, fetal B cell development also requires Ikaros. In post-natal Ikaros null mice, bone marrow (BM) lymphoid primed progenitors (LMPPs) are defective and their downstream common lymphoid progenitors (CLPs) are absent [15,29]. As CLPs give rise to all B lineage cells, B2 and B1 lymphocytes are missing in Ikaros null animals (Figure 3). Interestingly, CLPs are also reduced in patients with haploinsufficient germline IKZF1 mutations [9••].

The first *in vivo* demonstration that Ikaros plays a central role in B2 cell differentiation was performed with mice bearing the L/L hypomorphic mutation [30].