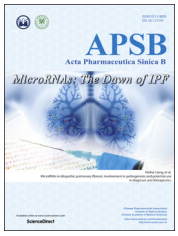
Acta Pharmaceutica Sinica B 2016;6(6):513–521

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

The Ikaros family of zinc-finger proteins

Yingzhi Fan, Duo Lu*

State Key Laboratory of Bioactive Substances and Function of Natural Medicine, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, China

Received 4 May 2016; received in revised form 20 May 2016; accepted 24 May 2016

KEY WORDS

Ikaros family;
Zinc-finger;
Lymphocyte development;
Drug target;
Transcription factor

Abstract Ikaros represents a zinc-finger protein family important for lymphocyte development and certain other physiological processes. The number of family members is large, with alternative splicing producing various additional isoforms from each of the five homologous genes in the family. The functional forms of Ikaros proteins could be even more diverse due to protein–protein interactions readily established between family members. Emerging evidence suggests that targeting Ikaros proteins is feasible and effective in therapeutic applications, although the exact roles of Ikaros proteins remain elusive within the intricate regulatory networks in which they are involved. In this review we collect existing knowledge as to the functions, regulatory pathways, and molecular mechanisms of this family of proteins in an attempt to gain a better understanding through the comparison of activities and interactions among family members.

*Corresponding author.
E-mail address: luduo@imm.ac.cn (Duo Lu).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2016.06.002

1. Introduction

Ikaros was first identified in 1992[1], where it displayed a strong phenotype in lymphocyte development. After over two decades of research, the situation became increasingly complex; four homologs were identified: Helios, Aiolos, Eos, and Pegasus[2], and each gene could produce several protein isoforms through alternative splicing. Interactions were common among isoforms and across family members, which presumably could generate a huge number of complexes through different combinations.

Through dimerization a set of two C2H2 zinc-fingers at the C-termini mediates protein–protein interactions within the family, and this highly conserved domain is present in most of the proteins in the family. Another common feature of the Ikaros proteins is an N-terminal domain composed of a maximum of four zinc-finger motifs for the recognition of target DNA sequences. The number of N-terminal fingers varies due to alternative splicing, and the isoforms without these fingers display a dominant negative effect in transcriptional activation[3].

Although their sequences have high similarity, the distribution of Ikaros family proteins varies. Ikaros, Helios, and Aiolos are mainly restricted to lymphoid cells and their progenitors at different developmental stages, despite the absence of Aiolos in hematopoietic stem cells (HSCs). In addition, Ikaros is also detected in brain, and both Ikaros and Helios are detected in erythroid cells. Eos and Pegasus are more widely expressed throughout body, including skeletal muscle, liver, brain, and heart. The highest Eos expression level is found in skeletal muscle. The detailed distribution of each member of Ikaros family is listed in Table 1 [2,4-6].

Existing knowledge of the Ikaros family indicates that these proteins are mainly involved in lymphocyte development, including a wide range of processes, such as apoptosis, cell cycle arrest, proliferation, and differentiation. Accordingly, failure in their proper regulation leads to various diseases such as cancer and autoimmunity.

More recently, Ikaros and Aiolos were found to be the main targets for immunomodulatory drug (IMiD)-induced cereblon (CRBN) ubiquitination and subsequent degradation in the treatment of multiple myeloma patients[7]. Inhibitors against casein kinase II (CK2) restored the ability of Ikaros to function as a tumor suppressor in high-risk leukemia[8]. Taken together, these findings suggest that targeting Ikaros family members was feasible and highly effective.

To develop different strategies for targeting Ikaros and its homologs, better understanding of the family is a prerequisite. In this review, we attempt to summarize the existing knowledge of the Ikaros family, including their functions, regulatory pathways, and molecular mechanisms of the regulation. Because there are far more studies on Ikaros, we discuss it separately from its homologs.

2. Ikaros

2.1. *Physiological functions*

Ikaros was first identified as an important protein for lymphocyte development from the study of a transgenic mouse model, and several mice models were generated thereafter to further investigate the function of Ikaros.

Homozygous deletion of exons 4 and 5 (originally called exons 3 and 4 without consideration of the noncoding exon 1) from Ikaros gene (*Ikzf1*), which encodes the first three N-terminal zinc-fingers, produced mice lacking T cells, B cells, natural killer (NK) cells, and

Table 1 Distribution of Ikaros family proteins.

Distribution[a]		Ikaros	Aiolos	Helios	Eos	Pegasus
Hematopoietic system	HSC	+	–	+	n.a.	n.a.
	CLP	+	+	+	n.a.	n.a.
	Pro-T cells	++	+	+	n.a.	n.a.
	DN T cells	++	+	+	n.a.	n.a.
	DP T cells	++	++	++	n.a.	n.a.
	SP T cells	++	++	+	n.a.	n.a.
	Activated T cells	++	++	+	n.a.	n.a.
	Pro-B cells	++	+	++	n.a.	n.a.
	Pre-B cells	++	++	+	n.a.	n.a.
	B cells (mature peripheral)	++	++	+	n.a.	n.a.
	Activated B cells	++	+++	+	n.a.	n.a.
	NK	+	+	+	n.a.	n.a.
	Erythroid precursors	+	n.a.	+	+	n.a.
	Myeloid precursors	+	n.a.	+	++	+
	Megakaryocytic cells	+	n.a.	n.a.	+++	+
Non-hematopoietic system	Developing nervous system	+	n.a.	n.a.	+	n.a.
	Brain	+	n.a.	n.a.	+	+
	Liver	n.a.	n.a.	n.a.	+	+
	Skeletal muscle	n.a.	n.a.	n.a.	++	+
	Kidney	n.a.	n.a.	n.a.	+	+
	Heart	n.a.	n.a.	n.a.	+	+

CLP, common lymphoid progenitors; DN, double negative; DP, double positive; HSC, hematopoietic stem cells; NK, natural killer cells; SP: single positive.

n.a.: not available.

[a]Note: data are not comparable between hematopoietic and non-hematopoietic systems.

their earliest defined progenitors, while the erythroid and myeloid lineages were intact[9]. Heterozygous animals also showed enlarged lymphoid organs and loss of NK cells[3].

Deletion of exon 8 led to the inactivation of Ikaros, with loss of the C-terminal fingers and a bipartite transcription activation domain. Homozygous mice with this mutation developed defects in lymphocytes, while fetal T lymphocytes, fetal and adult B lymphocytes, and their earliest progenitors were absent.

Individual deletion of exon 4 or 6 produced proteins lacking zinc-finger 1 or 4, respectively[10]. The transgenic mice had smaller amount of conventional B cells compared to wild-type animals. Mice lacking finger 1 had substantially decreased numbers of large pre-BII cells, while mice missing finger 4 had increased numbers of these cells. Both kinds of mice displayed fewer small pre-BII cells, immature B cells, and mature recirculating B cells, while deletion of finger 1 showed a more severe phenotype. Upon deletion of finger 4, mice displayed reduced $CD4^{-}CD8^{-}$ double-negative (DN) thymocytes, in contrast to a relatively normal number of DN population in the finger1-mutant mice. Detailed analysis suggested that the aberrant Ikaros led to a reduction of dynamic ranges of down-stream gene expression changes during thymocyte developmental transitions[11].

Conditional deletion of *Ikzf1* exon 5 in mice[12], which eliminates fingers 2 and 3 at the N-terminus, arrested pre-B cell differentiation at a stage with augmented proliferation and self-renewal signaling [mitogen-activated protein kinase (MAPK) pathway] and attenuated differentiation signaling (pre-B cell receptor pathway, preBCR pathway).

Apart from the exon deletion mutants, a knock-in mouse strain with a β-galactosidase in-frame insertion in *Ikzf1* exon 2 displayed reduced Ikaros function compared to the wild-type[13]. Fetal B cells were absent in the homozygous animals, but B cells nevertheless developed postnatally from a reduced pool of precursors.

Consistent with the results from mouse models displaying lymphocyte defects in development upon Ikaros mutation, clinical studies found genetic alterations in Ikaros strongly correlated to a poor outcome in high-risk acute lymphoblastic leukemia (ALL) patients. In Philadelphia chromosome-carrying ALL (*BCR-ABL1* ALL) patients, 83.7% patients had alterations in *IKZF1*, which resulted in haplo-insufficiency, expression of dominate negative isoform of Ikaros, or complete loss of Ikaros expression[14]. Recombination activating gene (*RAG*) was thought to be responsible for the deletion mutation of Ikaros in *BCR-ABL1* ALL patients. In another study which addressed young ALL patients without the Philadelphia chromosome, alteration in *IKZF1* was also found to be strongly associated with a poor clinic outcome[15].

2.2. *Regulatory pathways*

While phenotypes are most significant in lymphocyte differentiation, studies on signaling pathways in this area have attracted some attention. The preBCR signaling pathway has been extensively studied and produced a relatively clear picture of the regulatory network (Fig. 1). Upon preBCR activation, the coreceptors Igα/Igβ were phosphorylated by LYN, a SRC family protein-tyrosine kinase, followed by the recruitment and activation of spleen tyrosine kinase (SYK), which phosphorylated CD19 and B-cell phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) adaptor protein (BCAP). PI3K was consequently recruited to the cell membrane through the binding to CD19 and BCAP, and catalyzed the conversion of phosphatidylinositol (4,5) diphosphate [PI(4,5)P2] into phosphatidylinositol (3,4,5) triphosphate (PIP3), which was a favorite binding site for PH domain-containing signaling proteins such as protein kinase AKT, Bruton's tyrosine kinase (BTK) and phospholipase Cγ2 (PLCγ2). Membrane-bound AKT was activated through phosphorylation at T308 and S473, and subsequently translocated into the nucleus to phosphorylate transcription factor FOXO1, which was in turn exported out of nucleus and subjected to proteasome degradation. The decreased expression level of FOXO1 led to improper splicing of Ikaros mRNA[16].

While an activated preBCR pathway eventually reduces Ikaros activity, Ikaros counteracted the effect by projecting suppressions at two sites of the pathway. First, Ikaros repressed expression of the *Igll1* gene which encodes preBCR component $\lambda 5$[1]. Overexpression of Ikaros consistently led to reduction in the phosphorylation of SLP65, which was a substrate of SYK downstream of preBCR. SLP65 (also known as BLNK), activated by SYK phosphorylation, was a crucial protein for B-cell activation, which promoted apoptosis through the interaction with PLCγ2[17] and arrested the cell cycle through the inhibition of the JAK3/STAT5

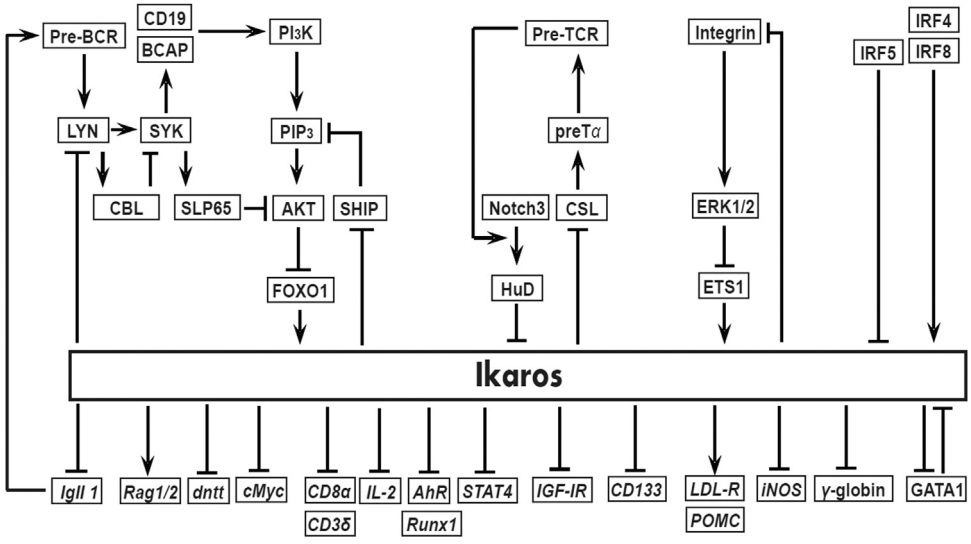


Figure 1 The regulation network of Ikaros.

pathway[18]. Secondly, the activity of the SRC kinase LYN was also inhibited by Ikaros, and subsequently restrained the abilities of E3 ligase CBL to ubiquitinylate SYK for proteasomal degradation and to interact with SLP65 by competing with PLCγ2[19]. Thus, Ikaros up-regulated SYK expression through the LYN/CBL pathway, but down-regulated SYK phosphorylation through the preBCR pathway. It is worth mentioning that SLP65 was suggested to inhibit the phosphorylation of AKT at S473 and promote FOXO1 stability[20]. This putatively should promote Ikaros activity, although existing data suggests that Ikaros mRNA level was not affected upon SLP65 reconstitution in SLP65-deficient pre-B cells[21].

Surprisingly, Ikaros was shown to down-regulate the expression of the inositol 5-phosphatase SHIP, which dephosphorylated the membrane component PIP3 at position 5 of the inositol ring. The modification on the membrane blocked the recruitment and subsequent activation of AKT, BTK and PLCγ2. Hence, Ikaros appeared to support AKT activation by downregulating SHIP, while Helios displayed the opposite function[22]. Collectively, an intricate network was built between Ikaros and preBCR, which included major nodes of LYN, CBL, SYK, SLP65, PI3K, SHIP, AKT and FOXO1.

Another relatively well-studied Ikaros-related pathway is the Notch pathway, which is important for tumor cell proliferation. A study of T-cell leukemogenesis demonstrated that in a pre-T cell receptor (preTCR)-dependent mode, Notch3 elevated the expression of the RNA binding protein HuD, which shifted the alternative splicing pattern of Ikaros towards the dominate-negative isoform[23]. Reciprocally, by competing with CSL (also known as RBP-Jk) at DNA binding sites in promoter regions, Ikaros counteracted the Notch effect on CSL activation and repressed the expression of downstream genes including the component of preTCR pre-Tα[24].

A recent study suggested that Ikaros was a target of the MAPK signaling pathway[25]: ERK1/2-mediated phosphorylation of ETS1 repressed its ability to up-regulate the expression of Ikaros. On the other hand, in the *Ikzf1* exon 5–deleted mice, elevated activity of ERK1/2 was observed, which correlated with a faster transit through the cell-cycle in large pre-B cells[12]. The augmented ERK1/2 activity was thought a result of the activated signaling pathway for integrin which was normally repressed by Ikaros in wild-type animals.

Ikaros was under the influence of several interferon regulatory factors (IRFs), which were important for B cell development and the inflammatory response of the immune system. Early study on pre-B cell development showed that IRF4 and 8 induced the expression of Ikaros and its homolog Aiolos, which worked together to inhibit preBCR expression and led to the cell-cycle withdrawal of small pre-B cells[26]. However, a more recent study on the B cell IgG2a/c isotype class-switch suggested that IRF8 but not IRF4 activated the *IKZF1* promoter, and IRF5 could inhibit such activation[27].

Post-translation modifications were detected on Ikaros. CK2 kinase was found to phosphorylate Ikaros at several sites and consequently lower it DNA affinity, while Protein Phosphatase 1 (PP1) had the ability to remove such modification[8]. SYK was also found to phosphorylate Ikaros but at different sites, which affected the nuclear localization of Ikaros[28]. Small ubiquitin-like modifier (SUMO)-ylation was detected on Ikaros[29], which disrupted Ikaros interactions with transcriptional corepressors such as Sin3A, Sin3B, Mi-2β and CtBP, and impaired the repressive activity of Ikaros. In this case, the nuclear localization of Ikaros was not affected. The process of SUMOylation was actively regulated by SUMO isopeptidases Senp1 and Axam and E3 ligases PIASx and PIAS3. Likewise, under the induction of IMiDs, Ikaros was ubiquitinylated by E3 ligase CRBN[7] and was subsequently degraded by proteasomes.

While Ikaros was targeted by many regulatory pathways, this transcription factor was shown to affect a large number of downstream proteins. Genome-wide analysis discovered thousands of DNA binding-sites for Ikaros[30], and many sites had been reported in individual studies. Unsurprisingly, many downstream genes were important for lymphocyte development, such as *dntt*[31] (encoding TdT) and *RAG* locus[32] for VDJ recombination, *CD8α* locus[33], *CD3δ*, *IL2*[34], *Ahr*, *Runx1*[35] and *STAT4*[36] for T cell differentiation, *IGF-IR* in T cell lymphoma[37], and *cMyc* in B cell differentiation[38], apart from those already mentioned above. In addition to lymphonoid genes, many hematopoietic genes, genes from the neuroendocrine system, and certain cancer markers were regulated by Ikaros, such as surface marker *CD133* of cancer stem cells[25], *LDL-R* and *POMC* in pituitary cells[39], *iNOS* in macrophages[40], γ-globin[41] and *GATA1* in primary megakaryocytes[42], while GATA1 was also found to repress Ikaros expression.

2.3. *Molecular mechanisms*

Ikaros functions through transcriptional regulation of its downstream proteins. Several levels of Ikaros-mediated transcriptional regulation have been proposed.

At the chromosome level, Ikaros was detected at discrete heterochromatin-containing foci in interphase nuclei, in complex with another family member, Helios[43], and transcriptionally inactive genes localized with centromeric Ikaros complexes in B cell nuclei[44]. This led to the suggestion that the transcription activity was compartmentalized, and Ikaros performed as a recruiter to bring target genes to a specific region. Further detailed analysis suggested that the DNA binding feature of Ikaros was closely correlated to the pericentromeric localization, and replacing the C-terminal zinc-finger region with a leucine-zipper dimerization motif did not appear to affect the formation of heterochromatin targeting[45].

At the nucleosomal level, Ikaros was found to associate with the nucleosome-remodeling and histone deacetylase (NuRD) complex to repress downstream genes[46]. The interaction between Ikaros and NuRD has been probed, and while the NuRD core element Mi-2β was thought to associate with the N- and C-terminal zinc-finger regions of Ikaros, another component of NuRD, HDAC2 was mapped onto a much wider region covering the previous two parts[47]. Another remodeling complex SWI/SNF was also detected in association with Ikaros, which was generally considered an activator for downstream genes. A weak but specific interaction was noticed between Ikaros and Brg-1 SWI/SNF ATPase[48].

At the RNA polymerase II (PolII) level, Ikaros regulated transcription initiation in association with C-terminal binding protein (CtBP) and CtBP-interacting protein (CtIP), which repressed transcription initiation through the interactions with general transcription factors TFIIB and TBP in the transcription pre-initiation complex. For example, Ikaros interacted with, retinoblastoma protein (Rb), CtBP and CtIP to repress the promoter of thymidine kinase[49], and the interaction between Ikaros and CtBP was also detected in the repression of CD133 expression[25]. A PEDLS sequence motif at Ikaros N-terminus was thought important for the interaction of CtBP[5].

Ikaros was also involved in the regulation of transcription elongation. By interacting with both CDK9 and PP1, Ikaros was proposed to transfer CDK9 to PP1α for dephosphorylation[50]. Consequently CDK9-containing positive transcription elongation factor b (P-TEFb) was prone to the dissociation from the inactive 7SK complex. The released P-TEFb gained access to PolII through association with gene regulatory region, where CDK9 phosphorylated the C-terminal domain (CTD) of PolII and transcription pausing factors NELF and DSIF and released PolII from the pausing phase into transcription elongation. Thus, through PP1/CDK9/CTD/NELF/DSIP, Ikaros promoted transcription elongation of PolII.

Ikaros was proposed to mediate crosstalk between different regulatory pathways, considering its simultaneous association with two functionally different protein machines. At β-globin loci of adult erythroid cells Ikaros was found in association with both NuRD (repressor) and SWI/SNF (activator) chromatin remodeling complexes[51]. A similar result was also found in proliferating T cells, while Aiolos was also detected in the NuRD containing complex[48]. The presence of two remodeling complexes was thought to repress γ-globin expression and facilitate the γ- to β-globin switch. Unfortunately no further investigation was carried out on the coordination between the two complexes. Similarly both P-TEFb and NuRD complexes were found in the same complex with Ikaros in a co-immunoprecipitation (coIP) experiment[50], though NuRD was also found to bind with Ikaros alone. While the association with the NuRD or NuRD-P-TEFb complex was Ikaros-dosage dependent, NuRD was thought to perform in the super complex as a regulatory component for transcription elongation.

3. Ikaros homologues

3.1. *Aiolos*

Within the Ikaros family, Aiolos is most closely related to Ikaros, which is reflected in their similar functions. Aiolos was found to have a role in the activation of B-cells, maturation of both B-cells and NK cells[52], differentiation of Th17 cells[53], and the generation of high affinity bone marrow plasma cells responsible for long-term immunity[54]. Misregulation of Aiolos was correlated to various diseases. Through the examination of unbiased massively parallel sequencing of whole exomes (WES), a recurrent mutation on Aiolos was recently identified as a putative cancer driver in chronic lymphocytic leukemia (CLL)[55]. Up-regulated expression of Aiolos has been noted in B-cell CLL (B-CLL)[56], non-Hodgkin lymphoma[57], lung cancer[58], and in peripheral blood mononuclear cell subsets from systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) patients[59]. In a mouse model, however, lack of Aiolos expression also led to an SLE-like phenotype[60]. Model cell lines used to study Aiolos include Nalm-6 ALL cells, Jurkat leukemia T-cells, and chicken DT40 lymphoma B-cells[61]. Overexpression of Aiolos was found to repress proliferation and apoptosis in Nalm-6 cells[62], but induced apoptosis in Jurkat cells[63]. Disruption of Aiolos expression in DT40 cells led to defects in gene conversion and the cells were prone to apoptosis.

Like Ikaros, Aiolos is under the regulation of the preBCR/SLP65 pathway. SLP65 reconstitution in SLP65-deficient pre-B cells led to elevated Aiolos expression[21], which was required for the appropriate silencing of the *Igll1* gene in cooperation with Ikaros during B-cell developmental transition from pre-BI to pre-BII stage. In addition, Ikaros, NF-κB, AP4, STAT3, AhR, and TGFβ were found to promote Aiolos expression[53,64,65]. IMiD-induced CRBN ubiquitinylation also targeted Aiolos, in addition to Ikaros, and led to its degradation. In reverse, a recent study suggested that Aiolos has the ability to repress the expression of CRBN component *CUL4A*, in association with another transcription factor Blimp1[66].

Interplay between Aiolos and cytokines has been widely studied. IL2 was found to promote tyrosine phosphorylation on Aiolos, which disrupted the interaction between Aiolos and Ras[5]. The released Aiolos stimulated the expression of Bcl-2, and eventually blocked apoptosis of T cells. IL4 was also found to promote the phosphorylation of Aiolos, which broke its interaction with Bcl-XL[67]. The released Bcl-XL in this case prevented T cell apoptosis (Fig. 2).

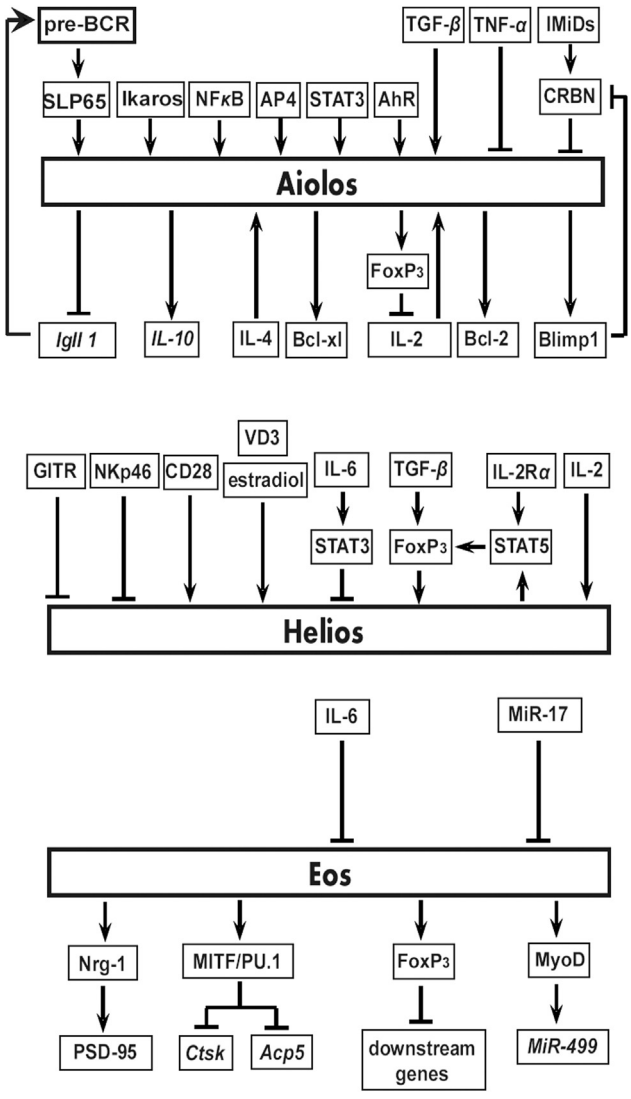


Figure 2 The regulation pathways of Aiolos, Helios, and Eos.

In addition to its anti-apoptotic ability, IL2 was thought to prevent Th17 differentiation, and Aiolos bound to the promoter of the *IL2* gene and repressed its activation[53]. Another study demonstrated that Aiolos achieved the down-regulation of *IL2* expression in physical association with another transcription factor, FoxP3[64]. TNFα may repress the expression of Aiolos,

while TNF-inhibitor (TNFi) showed the ability to promote Aiolos expression, and the enhanced Aiolos expression was found to correlate with a higher expression level of the anti-inflammation cytokine IL10 in Th17 cells[68]. Further study showed that Aiolos bound to conserved regions of the *IL10* locus and promoted expression of IL10[68]. The presence of IL10 reinforced the expression of itself through a yet unknown positive feedback loop, and IL1β had the ability to block the expression of IL10 in Th17 cells. Thus, Aiolos promoted Th17 differentiation, which contributed to inflammation, but also promoted the expression of anti-inflammation cytokine IL10 in Th17.

Interestingly, Th17 cells were found to differentiate preferentially from naïve FoxP3$^+$ Tregs in the presence of IL2 and IL1β[69], and the Helios$^-$FoxP3$^+$ subpopulation of Tregs also has IL17 or IL10 secreting cells[70]. This subset of Tregs can be isolated *ex vivo* based on the differential expression of IL-1RI and CCR7. Aiolos was expressed in this subset of Tregs, but not the Helios$^+$FoxP3$^+$ Treg subset, while Eos displayed an opposite expression pattern. The Helios$^-$FoxP3$^+$ Tregs are highly suppressive, and IL1β downregulated their suppressive capacity. Collectively there was a similarity between Th17 and this subset of Tregs, and it would be interesting to see whether Aiolos or other members of the Ikaros family contribute to the functional plasticity of Th17.

With respect to mechanism, Aiolos was found to disrupt the high-order chromatin structure that was necessary for long-range enhancer-promoter interactions of the *SHC1* gene, and consequently repressed the protein expression of a particular isoform of the adapter protein, p66^{Shc}, and led to anoikis resistance in lung cancer cells[58].

3.2. Helios

While early work suggested the involvement of Helios in leukemia, more recently Helios has been frequently proposed to function in Treg differentiation. Helios expression was considered a marker to distinguish thymotic Tregs from peripheral ones[71,72]. However, different opinions existed when Helios$^+$FoxP3$^+$ Tregs were also detected from the periphery[73]. In Tregs, expression of Helios was mostly correlated to the immunosuppressive activity[74], despite the fact that the percentage of Helios$^+$FoxP3$^+$ Tregs was found to be increased in active SLE patients[75], which was speculated to reflect an unsuccessful attempt to suppress the autoimmunity. In addition, exhausted CD4$^+$ T cells which arise during chronic infection were also found to have elevated Helios expression, but not Treg-marker FoxP3[76]. On the other hand, enhanced expression of Helios was correlated to the hyper-responsive NK cells[77].

Mouse models were created to investigate the functions of Helios. By deletion of exon 7 a Helios-null mouse was generated. The animal developed an RA-like phenotype with time, which was consistent with its function in Tregs to suppress inflammation[78]. To trace Helios expression *in vivo*, a transgenic mouse strain was generated with a variant green fluorescent protein Venus expressed as the reporter for Helios$^+$ cells[79]. Results from the Helios-reporter mice showed that the Helios$^+$ Treg population was superior in ability to suppress both the antigen-specific and TCR-stimulated T cell response, and inhibited the production of cytokines IFN-γ and IL-17.

Little is known about the regulatory pathway of Helios. Several cell surface proteins could lead to alteration in Helios expression. NKp46 reduction in NK cells was found to correlate to the enhanced expression level of Helios[77]. Antibody engagement of CD28 on $\gamma\delta$ T cell was found to elevate Helios expression[80]. Ligation of GITR to its antibody correlated with a drastic loss of Helios and FoxP3, a lower expression level of IL10, and a higher level of IFN-γ in intratumor Tregs[81].

A few exocellular reagents show the ability to affect Helios. The combination of Vitamin D3 and estradiol was found to induce Vdr$^+$CD4$^+$FoxP3$^+$Helios$^+$ T regulatory cells from Vdr$^+$CD4$^+$ T cells, and Helios expression was induced due to the action of calcitriol, the hormone form of Vitamin D3[82]. Cytokine IL2 administration was found to increase the ratio between FoxP3$^+$Helios$^+$ Tregs and FoxP3$^+$Helios$^-$ Tregs[83]. TGFβ promoted Helios expression in cooperation with FoxP3, while IL6 inhibited the expression of Helios in murine induced Tregs in a STAT3-dependent manner[84] (Fig. 2). Monocytes expressing IL12 blocked the proliferation of Helios$^+$ Tregs, while those expressing TNFα blocked proliferation of Helios$^-$ Tregs[85].

A detailed study on Helios regulation of downstream genes was restricted at FoxP3 only. Helios was found to bind to the promoter region of *FoxP3* and promote its expression[86]. However, a later study suggested that the effect on FoxP3 was possibly through the IL-2Rα-STAT5 pathway, in which a lower Helios level suppressed STAT5 activation, while activated STAT5 was required to promote expression of FoxP3 by binding to its promoter and CNS2 enhancer regions[78]. Enforced expression of STAT5 restored the expression of FoxP3 in Helios-deficient CD4$^+$ T cells, and prevented the expression of IFNγ.

3.3. Eos

Eos was first identified in the nervous system[4] and was found to associate with the Nrg-1 intracellular domain to promote the expression of postsynaptic density protein-95 (PSD-95), a scaffolding protein enriched in post-synaptic structures of neural cells[87]. Later studies found the involvement of Eos in a wide range of organic processes, such as cardiac and skeletal muscle development, osteoclast differentiation and lymphocyte differentiation.

Eos was found to bind directly to various proteins in the regulation of downstream genes. Eos associated with MyoD to regulate the *Myh7b/miR-499* gene in cardiac and skeletal muscle[88]. The complex of Eos, MITF and PU.1 was found to recruit CtBP and Sin3A to the promoter sites of *Ctsk* and *Acp5* and to repress their expression. Once Eos was removed from the promoter sites, the repression was released, which led to the initiation of osteoclast differentiation[89].

Eos functions in lymphocytes, mostly Treg and Th cells. In Tregs, Eos was found to interact with FoxP3 directly and induce chromatin modification and consequently downstream gene silencing[90]. During graft rejection, Eos expression was found to be reduced in Tregs[91]. Like Helios, IL6 was able to down-regulate Eos expression which led to the reprogramming of FoxP3$^+$ Tregs into helper-like T cells, without altered expression level of FoxP3[84,92]. The micro RNA miR-17 was found to target Eos and promote Th17 differentiation[93].

An Eos-null transgenic mouse strain was generated by deleting the last 3 exons, which led to undetectable expression of Eos mRNA and protein[94]. The mice nevertheless displayed normal numbers of Tregs with normal phenotypes which were fully competent suppressors. On the other hand, the Tconv cells from these mice had reduced amounts of IL2 secretion after TCR activation, and the animals developed more severe experimental autoimmune encephalomyelitis as compared to wild-type mice.

Structural studies were performed on Eos, including nuclear magnetic resonance (NMR) structures of the individual C-terminal zinc fingers[95] and an electron microscopy (EM) study on two C-terminal fingers of a fusion protein, MBP-tagged Eos[96]. The NMR study produced the structure of Eos C-terminal finger 2 (EosC2), but failed with EosC1; the homolog protein Pegasus C-terminal finger 1 (PegC1) structure was determined instead. Taking PegC1 structure as the reference, a homology model of EosC1 was generated. Structurally, PegC1 was in a canonical fold for zinc-fingers, while EosC2 was, surprisingly, in 2 conformations. Based on the EosC1 model and the structure of EosC2, the surface electrostatic potential analysis of Eos C-terminal fingers showed a mixed pattern of charges which was different from nucleic acid-binding fingers. Through *in vitro* biochemical assays, the Eos C-terminal 2-finger region was found to form a large homo-multimeric complex, which displayed a sphere shape by electron microscopy. However, the 3D structure was not pursued in the EM study.

3.4. *Pegasus*

Little is known on Pegasus. Upon its discovery in the year 2000, it was shown that Pegasus had the ability to interact with other homologs in the Ikaros family, and repressed expression of a reporter gene with its putative recognition element[4]. In 2011 a review article revealed an unpublished data where knockdown of Pegasus in zebra fish led to subtle effects in many blood cell lines[2]. The same group in 2013 reported a study on zebra-fish suggesting that Pegasus affected the left-right asymmetry during embryogenesis, and the effect was mediated through the transcriptional regulation of asymmetrical genes *pitx2*, *lefty2*, and *spaw*[97].

4. Concluding remarks

Ikaros-family proteins have a broad spectrum of functions that involves intricate signaling pathways and different levels of regulatory mechanisms. There are clear connections among family members. For example, Ikaros and Aiolos are both involved in the pathogenesis of leukemia, and both are targeted by IMiD-induced degradation. Treg marker FoxP3 is found in regulatory networks with Aiolos, Helios and Eos, while IL2 is connected to Ikaros, Aiolos and Helios. Obviously, members of Ikaros family proteins work together coordinately to conduct particular functions, and the mechanism behind the coordination is of interest to further investigations. In addition, while some Ikaros family proteins have been shown to be targetable by drugs, mainly through the manipulation of post-translation modifications at present, information on Ikaros proteins at the molecular level will provide more possibilities to target this biochemically interesting protein family.

References

1. Georgopoulos K, Moore DD, Derfler B. Ikaros, an early lymphoid-specific transcription factor and a putative mediator for T cell commitment. *Science* 1992;**258**:808–12.
2. John LB, Ward AC. The *Ikaros* gene family: transcriptional regulators of hematopoiesis and immunity. *Mol Immunol* 2011;**48**:1272–8.
3. Winandy S, Wu P, Georgopoulos K. A dominant mutation in the Ikaros gene leads to rapid development of leukemia and lymphoma. *Cell* 1995;**83**:289–99.
4. Perdomo J, Holmes M, Chong B, Crossley M. Eos and pegasus, two members of the Ikaros family of proteins with distinct DNA binding activities. *J Biol Chem* 2000;**275**:38347–54.
5. Schmitt C, Tonnelle C, Dalloul A, Chabannon C, Debré P, Rebollo A. Aiolos and Ikaros: regulators of lymphocyte development, homeostasis and lymphoproliferation. *Apoptosis* 2002;**7**:277–84.
6. Yoshida T, Georgopoulos K. Ikaros fingers on lymphocyte differentiation. *Int J Hematol* 2014;**100**:220–9.
7. Lu G, Middleton RE, Sun H, Naniong M, Ott CJ, Mitsiades CS, et al. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. *Science* 2014;**343**:305–9.
8. Dovat S, Song C, Payne KJ, Li Z. Ikaros, CK2 kinase, and the road to leukemia. *Mol Cell Biochem* 2011;**356**:201–7.
9. Georgopoulos K, Bigby M, Wang JH, Molnar A, Wu P, Winandy S, et al. The Ikaros gene is required for the development of all lymphoid lineages. *Cell* 1994;**79**:143–56.
10. Schjerven H, McLaughlin J, Arenzana TL, Frietze S, Cheng D, Wadsworth SE, et al. Selective regulation of lymphopoiesis and leukemogenesis by individual zinc fingers of Ikaros. *Nat Immunol* 2013;**14**:1073–83.
11. Arenzana TL, Schjerven H, Smale ST. Regulation of gene expression dynamics during developmental transitions by the Ikaros transcription factor. *Genes Dev* 2015;**29**:1801–16.
12. Joshi I, Yoshida T, Jena N, Qi X, Zhang J, Van Etten RA, et al. Loss of Ikaros DNA-binding function confers integrin-dependent survival on pre-B cells and progression to acute lymphoblastic leukemia. *Nat Immunol* 2014;**15**:294–304.
13. Kirstetter P, Thomas M, Dierich A, Kastner P, Chan S. Ikaros is critical for B cell differentiation and function. *Eur J Immunol* 2002;**32**:720–30.
14. Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J, et al. *BCR-ABL1* lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature* 2008;**453**:110–4.
15. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, et al. Deletion of *IKZF1* and prognosis in acute lymphoblastic leukemia. *N Engl J Med* 2009;**360**:470–80.
16. Alkhatib A, Werner M, Hug E, Herzog S, Eschbach C, Faraidun H, et al. FoxO1 induces Ikaros splicing to promote immunoglobulin gene recombination. *J Exp Med* 2012;**209**:395–406.
17. Yasuda T, Maeda A, Kurosaki M, Tezuka T, Hironaka K, Yamamoto T, et al. Cbl suppresses B cell receptor-mediated phospholipase C (PLC)-γ2 activation by regulating B cell linker protein-PLC-γ2 binding. *J Exp Med* 2000;**191**:641–50.
18. Nakayama J, Yamamoto M, Hayashi K, Satoh H, Bundo K, Kubo M, et al. BLNK suppresses pre-B-cell leukemogenesis through inhibition of JAK3. *Blood* 2009;**113**:1483–92.
19. Nera KP, Alinikula J, Terho P, Narvi E, Törnquist K, Kurosaki T, et al. Ikaros has a crucial role in regulation of B cell receptor signaling. *Eur J Immunol* 2006;**36**:516–25.
20. Ochiai K, Maienschein-Cline M, Mandal M, Triggs JR, Bertolino E, Sciammas R, et al. A self-reinforcing regulatory network triggered by limiting IL-7 activates pre-BCR signaling and differentiation. *Nat Immunol* 2012;**13**:300–7.
21. Thompson EC, Cobb BS, Sabbattini P, Meixlsperger S, Parelho V, Liberg D, et al. Ikaros DNA-binding proteins as integral components of B cell developmental-stage-specific regulatory circuits. *Immunity* 2007;**26**:335–44.
22. Alinikula J, Kohonen P, Nera KP, Lassila O. Concerted action of Helios and Ikaros controls the expression of the inositol 5-phosphatase SHIP. *Eur J Immunol* 2010;**40**:2599–607.
23. Bellavia D, Mecarozzi M, Campese AF, Grazioli P, Talora C, Frati L, et al. Notch3 and the Notch3-upregulated RNA-binding protein HuD regulate Ikaros alternative splicing. *EMBO J* 2007;**26**:1670–80.
24. Dumortier A, Jeannet R, Kirstetter P, Kleinmann E, Sellars M, dos Santos NR, et al. Notch activation is an early and critical event during T-cell leukemogenesis in Ikaros-deficient mice. *Mol Cell Biol* 2006;**26**:209–20.

25. Zhang L, Li H, Ge C, Li M, Zhao FY, Hou HL, et al. Inhibitory effects of transcription factor Ikaros on the expression of liver cancer stem cell marker CD133 in hepatocellular carcinoma. *Oncotarget* 2014;**5**:10621–35.
26. Ma S, Pathak S, Trinh L, Lu R. Interferon regulatory factors 4 and 8 induce the expression of Ikaros and Aiolos to down-regulate pre-B-cell receptor and promote cell-cycle withdrawal in pre-B-cell development. *Blood* 2008;**111**:1396–403.
27. Fang CM, Roy S, Nielsen E, Paul M, Maul R, Paun A, et al. Unique contribution of IRF-5-Ikaros axis to the B-cell IgG2a response. *Genes Immun* 2012;**13**:421–30.
28. Uckun FM, Ma H, Zhang J, Ozer Z, Dovat S, Mao C, et al. Serine phosphorylation by SYK is critical for nuclear localization and transcription factor function of Ikaros. *Proc Natl Acad Sci U S A* 2012;**109**:18072–7.
29. Gomez-del Arco P, Koipally J, Georgopoulos K. Ikaros SUMOylation: switching out of repression. *Mol Cell Biol* 2005;**25**:2688–97.
30. Ferreirós-Vidal I, Carroll T, Taylor B, Terry A, Liang Z, Bruno L, et al. Genome-wide identification of Ikaros targets elucidates its contribution to mouse B-cell lineage specification and pre-B-cell differentiation. *Blood* 2013;**121**:1769–82.
31. Trinh LA, Ferrini R, Cobb BS, Weinmann AS, Hahm K, Ernst P, et al. Down-regulation of TDT transcription in $CD4^{+}CD8^{+}$ thymocytes by Ikaros proteins in direct competition with an Ets activator. *Genes Dev* 2001;**15**:1817–32.
32. Reynaud D, Demarco IA, Reddy KL, Schjerven H, Bertolino E, Chen Z, et al. Regulation of B cell fate commitment and immunoglobulin heavy-chain gene rearrangements by Ikaros. *Nat Immunol* 2008;**9**:927–36.
33. Harker N, Naito T, Cortes M, Hostert A, Hirschberg S, Tolaini M, et al. The *CD8α* gene locus is regulated by the Ikaros family of proteins. *Mol Cell* 2002;**10**:1403–15.
34. Bandyopadhyay S, Duré M, Paroder M, Soto-Nieves N, Puga I, Macián F. Interleukin 2 gene transcription is regulated by Ikaros-induced changes in histone acetylation in anergic T cells. *Blood* 2007;**109**:2878–86.
35. Wong LY, Hatfield JK, Brown MA. Ikaros sets the potential for Th17 lineage gene expression through effects on chromatin state in early T cell development. *J Biol Chem* 2013;**288**:35170–9.
36. Yap WH, Yeoh E, Tay A, Brenner S, Venkatesh B. *STAT4* is a target of the hematopoietic zinc-finger transcription factor Ikaros in T cells. *FEBS Lett* 2005;**579**:4470–8.
37. Vishwamitra D, Curry CV, Alkan S, Song YH, Gallick GE, Kaseb AO, et al. The transcription factors Ik-1 and MZF1 downregulate *IGF-IR* expression in NPM-ALK^{+} T-cell lymphoma. *Mol Cancer* 2015;**14**:53.
38. Ma S, Pathak S, Mandal M, Trinh L, Clark MR, Lu R. Ikaros and Aiolos inhibit pre-B-cell proliferation by directly suppressing c-Myc expression. *Mol Cell Biol* 2010;**30**:4149–58.
39. Ezzat S, Asa SL. The emerging role of the Ikaros stem cell factor in the neuroendocrine system. *J Mol Endocrinol* 2008;**41**:45–51.
40. Cho SJ, Huh JE, Song J, Rhee DK, Pyo S. Ikaros negatively regulates inducible nitric oxide synthase expression in macrophages: involvement of Ikaros phosphorylation by casein kinase 2. *Cell Mol Life Sci* 2008;**65**:3290–303.
41. Bottardi S, Zmiri FA, Bourgoin V, Ross J, Mavoungou L, Milot E. Ikaros interacts with P-TEFb and cooperates with GATA-1 to enhance transcription elongation. *Nucleic Acids Res* 2011;**39**:3505–19.
42. Malinge S, Thiollier C, Chlon TM, Doré LC, Diebold L, Bluteau O, et al. Ikaros inhibits megakaryopoiesis through functional interaction with GATA-1 and NOTCH signaling. *Blood* 2013;**121**:2440–51.
43. Hahm K, Cobb BS, McCarty AS, Brown KE, Klug CA, Lee R, et al. Helios, a T cell-restricted Ikaros family member that quantitatively associates with Ikaros at centromeric heterochromatin. *Genes Dev* 1998;**12**:782–96.
44. Brown KE, Guest SS, Smale ST, Hahm K, Merkenschlager M, Fisher AG. Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin. *Cell* 1997;**91**:845–54.
45. Cobb BS, Morales-Alcelay S, Kleiger G, Brown KE, Fisher AG, Smale ST. Targeting of Ikaros to pericentromeric heterochromatin by direct DNA binding. *Genes Dev* 2000;**14**:2146–60.
46. Zhang J, Jackson AF, Naito T, Dose M, Seavitt J, Liu F, et al. Harnessing of the nucleosome-remodeling-deacetylase complex controls lymphocyte development and prevents leukemogenesis. *Nat Immunol* 2012;**13**:86–94.
47. Koipally J, Georgopoulos K. A molecular dissection of the repression circuitry of Ikaros. *J Biol Chem* 2002;**277**:27697–705.
48. Kim J, Sif S, Jones B, Jackson A, Koipally J, Heller E, et al. Ikaros DNA-binding proteins direct formation of chromatin remodeling complexes in lymphocytes. *Immunity* 1999;**10**:345–55.
49. Koipally J, Georgopoulos K. Ikaros–CtIP interactions do not require C-terminal binding protein and participate in a deacetylase-independent mode of repression. *J Biol Chem* 2002;**277**:23143–9.
50. Bottardi S, Mavoungou L, Pak H, Daou S, Bourgoin V, Lakehal YA, et al. The IKAROS interaction with a complex including chromatin remodeling and transcription elongation activities is required for hematopoiesis. *PLoS Genet* 2014;**10**:e1004827.
51. O'Neill DW, Schoetz SS, Lopez RA, Castle M, Rabinowitz L, Shor E, et al. An Ikaros-containing chromatin-remodeling complex in adult-type erythroid cells. *Mol Cell Biol* 2000;**20**:7572–82.
52. Holmes ML, Huntington ND, Thong RP, Brady J, Hayakawa Y, Andoniou CE, et al. Peripheral natural killer cell maturation depends on the transcription factor Aiolos. *EMBO J* 2014;**33**:2721–34.
53. Quintana FJ, Jin H, Burns EJ, Nadeau M, Yeste A, Kumar D, et al. Aiolos promotes T_H17 differentiation by directly silencing *Il2* expression. *Nat Immunol* 2012;**13**:770–7.
54. Cortes M, Georgopoulos K. Aiolos is required for the generation of high affinity bone marrow plasma cells responsible for long-term immunity. *J Exp Med* 2004;**199**:209–19.
55. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, et al. Mutations driving CLL and their evolution in progression and relapse. *Nature* 2015;**526**:525–30.
56. Duhamel M, Arrouss I, Merle-Beral H, Rebollo A. The Aiolos transcription factor is up-regulated in chronic lymphocytic leukemia. *Blood* 2008;**111**:3225–8.
57. Antica M, Cicin-Sain L, Kapitanovic S, Matulic M, Dzebro S, Dominis M. Aberrant Ikaros, Aiolos, and Helios expression in Hodgkin and non-Hodgkin lymphoma. *Blood* 2008;**111**:3296–7.
58. Li X, Xu Z, Du W, Zhang Z, Wei Y, Wang H, et al. Aiolos promotes anchorage independence by silencing $p66^{Shc}$ transcription in cancer cells. *Cancer Cell* 2014;**25**:575–89.
59. Cai X, Liu X, Du S, Xu X, Liu A, Ge X, et al. Overexpression of Aiolos in peripheral blood mononuclear cell subsets from patients with systemic lupus erythematosus and rheumatoid arthritis. *Biochem Genet* 2016;**54**:73–82.
60. Sun J, Matthias G, Mihatsch MJ, Georgopoulos K, Matthias P. Lack of the transcriptional coactivator OBF-1 prevents the development of systemic lupus erythematosus-like phenotypes in Aiolos mutant mice. *J Immunol* 2003;**170**:1699–706.
61. Narvi E, Nera KP, Terho P, Mustonen L, Granberg J, Lassila O. Aiolos controls gene conversion and cell death in DT40 B cells. *Scand J Immunol* 2007;**65**:503–13.
62. Zhuang Y, Li D, Fu J, Shi Q, Lu Y, Ju X. Overexpression of AIOLOS inhibits cell proliferation and suppresses apoptosis in Nalm-6 cells. *Oncol Rep* 2014;**31**:1183–90.
63. Zhuang Y, Lu Y, Li D, Sun N, Ju X. Upregulation of *AIOLOS* induces apoptosis and enhances etoposide chemosensitivity in Jurkat leukemia cells. *Oncol Rep* 2015;**33**:1319–25.
64. Gandhi R, Kumar D, Burns EJ, Nadeau M, Dake B, Laroni A, et al. Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and $Foxp3^{+}$ regulatory T cells. *Nat Immunol* 2010;**11**:846–53.
65. Ghadiri A, Duhamel M, Fleischer A, Reimann A, Dessauge F, Rebollo A. Critical function of Ikaros in controlling Aiolos gene expression. *FEBS Lett* 2007;**581**:1605–16.
66. Hung KH, Su ST, Chen CY, Hsu PH, Huang SY, Wu WJ, et al. Aiolos collaborates with Blimp-1 to regulate the survival of multiple myeloma cells. *Cell Death Differ* 2016;**23**:175–84.

67. Rebollo A, Ayllon V, Fleischer A, Martinez CA, Zaballos A. The association of Aiolos transcription factor and Bcl-xL is involved in the control of apoptosis. *J Immunol* 2001;**167**:6366–73.
68. Evans HG, Roostalu U, Walter GJ, Gullick NJ, Frederiksen KS, Roberts CA, et al. TNF-α blockade induces IL-10 expression in human $CD4^+$ T cells. *Nat Commun* 2014;**5**:3199.
69. Valmori D, Raffin C, Raimbaud I, Ayyoub M. Human $ROR\gamma t^+$ T_H17 cells preferentially differentiate from naive $FOXP3^+$ Treg in the presence of lineage-specific polarizing factors. *Proc Natl Acad Sci U S A* 2010;**107**:19402–7.
70. Raffin C, Pignon P, Celse C, Debien E, Valmori D, Ayyoub M. Human memory $Helios^-$ $FOXP3^+$ regulatory T cells (Tregs) encompass induced Tregs that express Aiolos and respond to IL-1β by downregulating their suppressor functions. *J Immunol* 2013;**191**:4619–27.
71. Shevach EM, Thornton AM. tTregs, pTregs, and iTregs: similarities and differences. *Immunol Rev* 2014;**259**:88–102.
72. Singh K, Hjort M, Thorvaldson L, Sandler S. Concomitant analysis of Helios and Neuropilin-1 as a marker to detect thymic derived regulatory T cells in naive mice. *Sci Rep* 2015;**5**:7767.
73. Szurek E, Cebula A, Wojciech L, Pietrzak M, Rempala G, Kisielow P, et al. Differences in expression level of Helios and Neuropilin-1 do not distinguish thymus-derived from extrathymically-induced $CD4^+Foxp3^+$ regulatory T cells. *PLoS One* 2015;**10**:e0141161.
74. Ross EM, Bourges D, Hogan TV, Gleeson PA, van Driel IR. Helios defines T cells being driven to tolerance in the periphery and thymus. *Eur J Immunol* 2014;**44**:2048–58.
75. Golding A, Hasni S, Illei G, Shevach EM. The percentage of $FoxP3^+Helios^+$ Treg cells correlates positively with disease activity in systemic lupus erythematosus. *Arthritis Rheum* 2013;**65**:2898–906.
76. Crawford A, Angelosanto JM, Kao C, Doering TA, Odorizzi PM, Barnett BE, et al. Molecular and transcriptional basis of $CD4^+$ T cell dysfunction during chronic infection. *Immunity* 2014;**40**:289–302.
77. Narni-Mancinelli E, Jaeger BN, Bernat C, Fenis A, Kung S, De Gassart A, et al. Tuning of natural killer cell reactivity by NKp46 and Helios calibrates T cell responses. *Science* 2012;**335**:344–8.
78. Kim HJ, Barnitz RA, Kreslavsky T, Brown FD, Moffett H, Lemieux ME, et al. Stable inhibitory activity of regulatory T cells requires the transcription factor Helios. *Science* 2015;**350**:334–9.
79. Sugita K, Hanakawa S, Honda T, Kondoh G, Miyachi Y, Kabashima K, et al. Generation of Helios reporter mice and an evaluation of the suppressive capacity of $Helios^+$ regulatory T cells *in vitro*. *Exp Dermatol* 2015;**24**:554–6.
80. Peters C, Oberg HH, Kabelitz D, Wesch D. Phenotype and regulation of immunosuppressive Vδ2-expressing $\gamma\delta$ T cells. *Cell Mol Life Sci* 2014;**71**:1943–60.
81. Schaer DA, Budhu S, Liu C, Bryson C, Malandro N, Cohen A, et al. GITR pathway activation abrogates tumor immune suppression through loss of regulatory T cell lineage stability. *Cancer Immunol Res* 2013;**1**:320–31.
82. Spanier JA, Nashold FE, Mayne CG, Nelson CD, Hayes CE. Vitamin D and estrogen synergy in *Vdr*-expressing $CD4^+$ T cells is essential to induce $Helios^+FoxP3^+$ T cells and prevent autoimmune demyelinating disease. *J Neuroimmunol* 2015;**286**:48–58.
83. Elkord E, Sharma S, Burt DJ, Hawkins RE. Expanded subpopulation of $FoxP3^+$ T regulatory cells in renal cell carcinoma co-express Helios, indicating they could be derived from natural but not induced Tregs. *Clin Immunol* 2011;**140**:218–22.
84. Takatori H, Kawashima H, Matsuki A, Meguro K, Tanaka S, Iwamoto T, et al. Helios enhances Treg cell function in cooperation with FoxP3. *Arthritis Rheumatol* 2015;**67**:1491–502.
85. Zhong H, Yazdanbakhsh K. Differential control of $Helios^{+/-}$ Treg development by monocyte subsets through disparate inflammatory cytokines. *Blood* 2013;**121**:2494–502.
86. Getnet D, Grosso JF, Goldberg MV, Harris TJ, Yen HR, Bruno TC, et al. A role for the transcription factor Helios in human $CD4^+CD25^+$ regulatory T cells. *Mol Immunol* 2010;**47**:1595–600.
87. Bao J, Lin H, Ouyang Y, Lei D, Osman A, Kim TW, et al. Activity-dependent transcription regulation of PSD-95 by neuregulin-1 and Eos. *Nat Neurosci* 2004;**7**:1250–8.
88. Yeung F, Chung E, Guess MG, Bell ML, Leinwand LA. *Myh7b/miR-499* gene expression is transcriptionally regulated by MRFs and Eos. *Nucleic Acids Res* 2012;**40**:7303–18.
89. Hu R, Sharma SM, Bronisz A, Srinivasan R, Sankar U, Ostrowski MC. Eos, MITF, and PU.1 recruit corepressors to osteoclast-specific genes in committed myeloid progenitors. *Mol Cell Biol* 2007;**27**:4018–27.
90. Bettini ML, Pan F, Bettini M, Finkelstein D, Rehg JE, Floess S, et al. Loss of epigenetic modification driven by the Foxp3 transcription factor leads to regulatory T cell insufficiency. *Immunity* 2012;**36**:717–30.
91. Campos-Mora M, Morales RA, Pérez F, Gajardo T, Campos J, Catalan D, et al. $Neuropilin\text{-}1^+$ regulatory T cells promote skin allograft survival and modulate effector $CD4^+$ T cells phenotypic signature. *Immunol Cell Biol* 2015;**93**:113–9.
92. Sharma MD, Huang L, Choi JH, Lee EJ, Wilson JM, Lemos H, et al. An inherently bifunctional subset of $Foxp3^+$ T helper cells is controlled by the transcription factor Eos. *Immunity* 2013;**38**:998–1012.
93. Liu SQ, Jiang S, Li C, Zhang B, Li QJ. miR-17-92 cluster targets phosphatase and tensin homology and Ikaros family zinc finger 4 to promote T_H17-mediated inflammation. *J Biol Chem* 2014;**289**:12446–56.
94. Rieder SA, Metidji A, Glass DD, Thornton AM, Ikeda T, Morgan BA, et al. Eos is redundant for regulatory T cell function but plays an important role in IL-2 and Th17 production by $CD4^+$ conventional T cells. *J Immunol* 2015;**195**:553–63.
95. Westman BJ, Perdomo J, Matthews JM, Crossley M, Mackay JP. Structural studies on a protein-binding zinc-finger domain of Eos reveal both similarities and differences to classical zinc fingers. *Biochemistry* 2004;**43**:13318–27.
96. Westman BJ, Perdomo J, Sunde M, Crossley M, Mackay JP. The C-terminal domain of Eos forms a high order complex in solution. *J Biol Chem* 2003;**278**:42419–26.
97. John LB, Trengove MC, Fraser FW, Yoong SH. Ward AC. Pegasus, the 'atypical' Ikaros family member, influences left-right asymmetry and regulates *pitx2* expression. *Dev Biol* 2013;**377**:46–54.