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Helios Deficiency Has Minimal Impact on T Cell Development and Function

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Helios is a member of the Ikaros family of zinc finger transcription factors. It is expressed mainly in T cells, where it associates with Ikaros-containing complexes and has been proposed to act as a rate-limiting factor for Ikaros function. Overexpression of wild-type or dominant-negative Helios isoforms profoundly alters αβ T cell differentiation and activation, and endogenous Helios is expressed at strikingly high levels in regulatory T cells. Helios has also been implicated as a tumor suppressor in human T cell acute lymphoblastic leukemias. These studies suggest a central role for Helios in T cell development and homeostasis, but whether this protein is physiologically required in T cells is unclear. We report herein that inactivation of the Helios gene by homologous recombination does not impair the differentiation and effector cell function of αβ and γδ T cells, NKT cells, and regulatory T cells. These results suggest that Helios is not essential for T cells, and that its function can be compensated for by other members of the Ikaros family. The Journal of Immunology, 2009, 183: 2303–2311.

The Helios transcription factor (Ikzf2) is a member of the Ikaros family of zinc finger regulators that includes Ikaros (Ikarzf1), Aiolos (Ikarzf3), Eos (Ikarzf4), and Pegasus (Ikarzf5). Ikaros family proteins share a similar structure that is characterized by highly conserved zinc finger domains at the N and C termini (1). Four N-terminal zinc fingers are responsible for DNA binding to consensus target sequences, while two C-terminal zinc fingers mediate homo- and heterodimerization between family members (2–8). Ikaros, Helios, and Aiolos all interact with the nucleosome remodeling and deacetylase (NuRD) histone deacetylase complex (9, 10), suggesting that they play pivotal roles in chromatin remodeling at their target genes.

Ikaros and Aiolos are critical regulators of hematopoiesis. Ikaros is implicated in stem cell renewal, fetal and adult erythropoiesis, and dendritic cell (DC)3 differentiation (11–14). Ikaros and Aiolos perform distinct functions in B cells. Aiolos is essential for many aspects of B cell differentiation and function, including B cell proliferation, marginal zone vs follicular B cell fate choice, and the development of high-affinity plasma cells (15–17). Ikaros controls early steps of B cell differentiation, including commitment, as well as Ig class switch recombination in mature B cells (18–21). Additionally, both Ikaros and Aiolos are required to limit B cell proliferation in response to activation (15, 18). Thus, Ikaros and Aiolos appear to play mostly distinct, but also overlapping, roles in B cells.

In T cells, Ikaros appears to be singularly important. Ikaros deficiency leads to absence of fetal T cell development, while postnatal T cell differentiation is associated with enhanced pre-TCR signaling, leading to increased proliferation of DN4 thymocytes (14, 22). Loss of Ikaros also leads to a decreased γδ T cell pool, as well as altered commitment to the CD4 and CD8 lineages (14, 23, 24). In mature T cells, Ikaros appears to suppress Th1 polarization (25) and to limit proliferation in response to signaling in both CD4+ and CD8+ T cells (26). Finally, Ikaros is involved in silencing Notch signaling during the double-negative to double-positive transition, a function that is likely to contribute to its tumor suppressor function in this lineage (27). Indeed, Ikaros deficiency is strongly associated with development of T cell leukemias that exhibit high levels of Notch activation (28, 29). The multiple abnormalities seen in Ikaros-deficient T cells contrast with the largely normal T cell compartment in Aiolos-deficient mice. However, Aiolos-null T cells also hyperproliferate to activation signals (15), suggesting that, as in B cells, both Ikaros and Aiolos are required to set the threshold for the proliferative response of these cells to activation.

Helios is conspicuous for its high expression from the earliest stages of T cell development (5, 30). Strikingly, Helios is induced >10-fold in CD4+Foxp3+ regulatory T (Treg) cells (31–33). Its expression in Treg cells does not require Foxp3, a transcriptional regulator essential for Treg cell differentiation (34), suggesting that Helios might function as an upstream regulator of Foxp3, or perhaps define a parallel transcriptional circuit in these cells. Helios is not expressed in mature B cells, DCs, or myeloid cells. At the molecular level, Helios associates with a subset of Ikaros complexes that localize near centromeric heterochromatin in T cells (5), suggesting that it might act as a rate-limiting factor of Ikaros function. Gain-of-function studies, using full-length or dominant-negative (dn) Helios lacking the DNA-binding domain, suggest a key role for this protein in T cell differentiation and function.
Overexpression of full-length Helios blocks αβ T cell differentiation at the CD4+CD8− stage in the thymus, and it results in increased frequencies of γδ T cells and NK cells in peripheral lymphoid organs, while overexpression of ΔN Helios leads to increased T cell proliferation upon TCR stimulation and the development of T lymphomas (35). Furthermore, ΔN Helios isoforms or allelic loss have been detected in some human T-acute lymphoblastic leukemias or T cell lymphomas (36–39). These results suggest that Helios is an essential regulator of T cell homeostasis and a tumor suppressor.

While these studies have been informative, they are unclear, as overexpression of either full-length or ΔN proteins must be interpreted cautiously since they cannot inhibit the normal function of related endogenous proteins. This is especially true for the Ikaros family, as four of its five members (Ikaros, Alios, Helios, and Eos) are coexpressed in T cells. Thus, it is important to understand the exact role of each protein in target gene activation/repression and T cell development. At the present time, Helios function remains unknown.

In this study, we investigated Helios function in T cells by generating a null mutation for this gene by homologous recombination. We find that Helios is not essential for T cell differentiation, homeostasis, and function, and that Helios-deficient mice do not develop T cell malignancies.

Materials and Methods

Generation of Helios-null mice

The Helios targeting vector is depicted in Fig. 1A. The C-terminal part of Helios exon 7 was replaced by a 1.8-kb floxed PGK-neo-poly(A) cassette between the indicated SalI and XbaI sites. The vector was transfected into P1 TCry embryonic stem cells, and homologous recombination events were detected by Southern blot using external probes A and B with BamHI-digested genomic DNA. Two embryonic stem cell clones, MBA120 and MBA93, were identified as positive and injected into C57BL/6 (B6) blastocysts to produce chimeric mice. Germine transmission was verified by PCR on tail DNA using primers P3 and P4 to detect the wild-type (WT) allele, and P3 and P5 to detect the mutant allele. Mice derived from both clones gave similar phenotypes. The experiments described in this paper were performed using mice generated from clone MBA120 and backcrossed once onto the B6 background.

RT-PCR, real-time RT-PCR, and primers

For RT-PCR and real-time PCR, RNA was extracted using the RNeasy Mini kit (Qiagen) and reverse transcribed with SuperScript II reverse transcriptase (Invitrogen). mRNA levels for Alios, Helios, Eos, and Ikaros were quantified by real-time PCR using SYBR Green JumpStart Mini kit (Qiagen) and reverse transcribed with SuperScript II reverse transcriptase. Real-time RT-PCR was performed using a Foxp3 staining kit (eBioscience). Cells were analyzed on a FACSCalibur (BD Biosciences), and data were analyzed with FlowJo software (Tree Star). Sorting was performed on a FACS Vantage SE option DiVa (BD Biosciences). Sort purity was >95%.

Proliferation assays

To induce the proliferation of peripheral CD4+ and CD8+ T cells, whole splenic CD4+ and CD8+ T cells, as well as splenic CD4+CD45+CD25+ and CD8+CD45+ T cells, were sorted, incubated with CFSE, and then seeded into 96-well plates coated with anti-CD3 (10 μg/ml; eBioscience) or anti-CD3 (10 μg/ml) plus anti-CD28 (5 μg/ml; eBioscience) in complete RPMI medium (RPMI 1640 supplemented with 10% FCS, 25 mM HEPES, 2 mM l-glutamine, 1 × nonessential amino acids, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, and 1% penicillin in 96-well plates) for 3 days. Cells were cultured without Ab stimulation. Cells were cultured at 2.5 × 10^5 cells per well for the anti-CD3 stimulation and unstimulated controls, and at 1 × 10^5 cells per well for anti-CD3 plus anti-CD28.

For Treg cell assays, Treg (CD4+CD25+CD44+), Th (CD4+CD25−CD44+), and APCs (Thy1.2+) were sorted from pooled spleen and lymph node cells. Treg or Th cells were incubated with CFSE and cultured in complete RPMI medium in 96-well plates at 5 × 10^3 cells per well in 96-well plates coated with anti-CD3 (1μg/ml) and 10^3 APCs, in the absence or presence of recombinant human IL-2 (100 U/ml). Cells were harvested after 3 days and stained for CD4 and CD25 expression before analysis.

Th1 and Th2 induction

Th1 and Th2 polarizing cultures were performed mostly according to Tu et al. (41). Briefly, CD4+CD45+CD25+ Th cells and Thy1.2+ APCs were sorted from adult spleens. In neutral conditions, 25 × 10^5 Th cells were cultured with 25 × 10^3 APCs (mitomycin C treated), recombinant human IL-2 (10 U/ml; PeproTech), soluble anti-CD3 (0.1 μg/ml), and anti-CD28 (0.5 μg/ml) in 96-well plates in triplicate samples. In Th1 polarizing conditions, anti-IL-4 (10 μg/ml; eBioscience) and recombinant murine IL-12 (5 ng/ml; eBioscience) were added to these cultures. Th2 polarizing conditions, anti-IL-12 (10 μg/ml; eBioscience) and recombinant IL-4 (20 ng/ml; PeproTech) were added to the cultures. After 7 days of culture, cells were restimulated with PMA (50 ng/ml; Sigma-Aldrich) and ionomycin (500 ng/ml; Sigma-Aldrich) for 4 h. GolgiPlug (brefeldin A; BD Biosciences) was added at 1 μl/ml for the last 2 h. Cells were harvested and stained to assess surface Thy1.2, CD4, and intracellular IL-4 and IFN-γ. Intracellular staining was performed using the Cytofix/Cytoperm permeabilization kit (BD Biosciences).

Detection of perforin and granzyme B

Splenic T cells were purified by negative selection using Abs specific for B220, CD11b, CD11c, Ter-119, and DX5. T cells (25 × 10^6/well) were then cultured in complete RPMI medium with or without recombinant IL-2 at 100 U/ml and anti-CD28. After 3 days, cells were harvested and stained for CD4 and CD8 before intracellular staining for granzyme B and perforin with a Foxp3 staining buffer system (eBioscience).
Results

We produced a Helios-null mutation by targeting a PGK-neo cassette into exon 7 of the Helios locus by homologous recombination (Fig. 1A). This mutation deletes most of exon 7, including the sequences that encode the C-terminal zinc fingers, as indicated by Southern blot analyses of mutant embryonic stem cells and PCR analyses of tail DNA from heterozygote and homozygote Helios mice (Fig. 1A and C). Helios protein is likely to be nonspecific since it was not detected with Ab2. Western blot of WT and Helios−/− thymocyte nuclear extracts using the Abs Ab1 and Ab2. TATA-box binding protein (TBP) was detected as a loading control. Note that the 50-kDa polypeptide detected in both WT and He−/− cells is likely to be nonspecific since it was not detected with Ab2.

Although homozygote Helios−/− and heterozygote Helios+/− animals were born at Mendelian frequencies and were of normal body size at birth (data not shown), many Helios−/− pups died within the first weeks of postnatal life (Fig. 2A). This early lethality increased as Helios−/− mice were backcrossed onto the B6 background, and no surviving homozygotes could be obtained after 10 generations of backcrossing. We thus studied the phenotype of Helios mutants on a mixed 129/Sv:B6 background. It is unclear why so many Helios−/− mutants die after birth. However, this is probably not associated with defective hematopoiesis, as 14.5 days postcoitus Helios−/− fetal liver cells efficiently reconstituted all hematopoietic lineages after adoptive transfer into lethally irradiated mice (data not shown). Surviving adult Helios−/− mice,
particularly females, were markedly smaller than WT or heterozygote littersmates in size and weight (Fig. 2, B and C, and data not shown). Helios−/− animals remained smaller as they aged, but these differences were no longer statistically significant in the male population (Fig. 2C). Helios−/− mice lived to at least 22 mo of age and showed no overt signs of ill health (nine mice analyzed between 16 and 22 mo of age; data not shown). Helios−/− mice exhibited smaller eye-openings, a phenotype that might be linked to an abnormal growth of eyelids (data not shown). Both male and female Helios−/− mice were fertile. These results indicate that Helios−/− mice do not spontaneously develop health-threatening illness if they survive the weaning period.

To address the role of Helios in αβ T cell development, we analyzed T cell populations in the thymuses and spleens of WT and Helios−/− mice. Immature CD4−CD8−CD3− double-negative thymocytes were analyzed for CD44 and CD25 expression, while the more mature CD4+CD8+ double-positive thymocytes and the CD4+CD8− and CD4−CD8+ single-positive cells were analyzed for CD3, αβTCR, CD24, and CD69 expression. All double-negative subpopulations (CD44+CD25−, CD44+CD25+, CD44−CD25+, and CD44−CD25−) were similar in frequency between WT and mutant thymocytes from adult mice (7–9 wk old) and newborn animals (1 day old) (Fig. 3A–D). Double-positive and single-positive thymocyte populations were also similar in frequencies and absolute numbers according to their CD4 and CD8 profiles, and no differences were observed in terms of CD3, αβTCR, CD24, and CD69 expression for each population (data not shown). Additionally, peripheral CD4+ and CD8+ T cells from the lymph nodes and spleens were comparable in frequency between WT and mutant mice (Fig. 3E), and no differences were observed in CD62L and CD44 expression in these cell types (data not shown), suggesting that memory T cell populations were unaltered. These results indicate that αβ T cell differentiation and homeostasis proceeds normally in the absence of Helios.

NKT cells, γδ T cells, and Treg cells also differentiate in the thymus, and these cells express high levels of Helios mRNA (Refs. 30, 32, 33 and data not shown). We therefore analyzed these cell types in the thymus and spleen of Helios−/− mice. γδ T cell and NKT cell (CD3 DX5+) frequencies in the mutant organs were similar to those from WT animals (Fig. 4, A and B). Helios−/− animals also showed normal percentages of thymic and splenic CD25+Foxp3+ Treg cells (Fig. 4C). Furthermore, the above cell types differentiated normally when analyzed in competitive mixed bone marrow chimera experiments where WT and Helios−/− bone marrow cells were transferred to lethally irradiated WT recipients (data not shown). These results indicate that γδ T cells, NKT cells, and Treg cells mature normally in Helios-deficient mice.

To determine whether loss of Helios affects mature T cell function, we first analyzed the capacity of Helios−/− peripheral CD4+ and CD8+ T cells to proliferate in response to TCR stimulation in vitro, as Ikaros and Aiolos have been shown to limit the proliferation threshold of T and B cells upon activation (14, 15, 18, 26, 28). Naïve CD4+CD25−CD44− and CD8+CD44− T cells, as well as whole CD4+ and CD8+ T cells, were sorted from WT and Helios−/− spleens, labeled with CFSE, and cultured for 3 days with anti-CD3 Abs alone or in combination with anti-CD28. These experiments show that WT cells and Helios−/− CD4+ and CD8+ T cells proliferate similarly to TCR stimulation in the absence or presence of costimulatory signals (Fig. 5A).

To test if Helios−/− CD4+ T cells efficiently differentiate into Th1 or Th2 cells, we measured the capacity of these cells to produce IFN-γ and IL-4 under neutral and polarizing conditions. In neutral conditions, CD4+CD25−CD44− splenic T cells were cultured with Thy1.2+ APCs in the presence of anti-CD3 and anti-CD28 Abs, as well as IL-2, for 7 days to evaluate the capacity of these cells to produce cytokines upon stimulation. In Th1 polarizing conditions, CD4+CD25−CD44− splenic T cells were cultured as above in the presence of IL-12 and anti-IL-4 Abs. In Th2 polarizing conditions, the same cells were cultured as above in the presence of IL-4 and anti-IL-12 Abs. After 7 days, all cultures were restimulated with PMA and ionomycin for 4 additional hours before analysis by intracytoplasmic staining. As shown in Fig. 5B, Helios−/− CD4+ T cells differentiated into IFN-γ Th1 and IL-4+ Th2 cells as efficiently as did WT CD4+ T cells in all conditions, indicating that CD8+ T cell function is unaffected in Helios−/− mice.

To evaluate if Helios−/− CD8+ T cells function as mature effector cells, we tested their capacity to produce perforin and granzyme B, two molecules important for cytotoxic function, upon stimulation. Splenic T cells were enriched by negative depletion and cultured with IL-2 for 3 days. CD8+ T cells were analyzed for the expression of perforin and granzyme B by intracellular staining. Comparable percentages of CD8+ T cells were induced to express perforin and granzyme B in both WT and Helios−/− cultures (Fig. 5C), suggesting that CD8+ T cell function is normal in Helios−/− mice.

Since Helios expression is strongly induced in Treg cells, we asked if Helios deficiency specifically affects Treg cell function. We first tested if Helios contributes to the low proliferative response of Treg cells to TCR-induced signals. As depicted in Fig. 6A, neither WT nor Helios−/− Treg cells (CD4+CD25+CD44−), purified from lymph nodes and spleens, responded to anti-CD3 stimulation in a 3-day coculture assay with APCs. Additionally, both WT and Helios−/− Treg cells proliferated similarly when stimulated with anti-CD3 Abs in the presence of IL-2, although the
proportion of Helios−/− Treg cells that responded to this stimulation was reduced by about one-third. Thus, Helios−/− Treg cells do not exhibit an altered pattern of proliferation under established conditions. We next evaluated if Helios−/− Treg cells could suppress the proliferation of responder Th (CD4+CD25+) cells stimulated with anti-CD3 Abs and APCs. Helios−/− Treg cells suppressed Th cell proliferation as efficiently as did WT Treg cells (Fig. 6B). Collectively, these results indicate that mature Treg cells function normally in the absence of Helios.

Lastly, we investigated the possibility that Helios−/− T cells might compensate for loss of Helios activity by up-regulating the expression of other Ikaros family members. We tested the mRNA levels of Aiolos, Ikaros, Eos, and Helios in WT and Helios−/− thymocytes by real-time RT-PCR (Fig. 7A). Helios−/− thymocytes exhibited a small but statistically significant increase in Aiolos mRNA levels (WT, 0.97 ± 0.03; Helios−/−, 1.26 ± 0.09), while Ikaros and Eos mRNA levels were similar between genotypes. At the protein level, the expression of Aiolos and Ikaros was

FIGURE 3. αβ T cell development or homeostasis in Helios−/− mice. Thymocytes from WT and Helios−/− adult mice (A and B) and neonates (C and D) were analyzed for expression of the indicated markers. Numbers in the contour plots indicate percentages of the gated cells. Bar graphs indicate mean absolute numbers of the different thymocyte populations analyzed ± SD, as calculated from four independent experiments. E, WT and Helios−/− splenocytes and lymph node cells were analyzed for CD4 and CD8 expression. Results are representative of three similar experiments.
comparable between WT and mutant thymocytes (Fig. 7B). These data suggest that Helios−/− T cells do not compensate for Helios deficiency by drastically up-regulating Aiolos and Ikaros expression. Furthermore, Helios mRNA levels remained similar between WT and mutant thymocytes despite loss of Helios at the protein level, suggesting that Helios does not autoregulate its own transcription.

Discussion
We report herein the generation of the first Helios-deficient mouse line. Our data show that Helios is essential for the first weeks of life, as most Helios−/− mice (100% on a pure B6 background) die during this period. The physiological functions that depend strictly on Helios remain unknown, but they are unlikely to be related to hematological or immune defects. Helios expression outside of the hematopoietic system is poorly characterized at present. Together with reports showing that Ikaros controls neural and endocrine functions (42–44), the severe viability defect of Helios−/− mice provides further evidence that Ikaros family members exert important functions beyond the hematopoietic system.

In mature hematopoietic cells, Helios expression is restricted to T cells (5, 30), suggesting that Helios may control important aspects of T cell differentiation and/or function. We show herein that Helios is not essential for the development, homeostasis, and...
function of thymic-derived T lymphocytes, in contrast to expectations from earlier overexpression studies suggesting a primary role for this transcription factor in /H9251/H9252 T cell development and as a tumor suppressor. In particular, most of the defects associated with Ikaros deficiency are not detected in Helios-null mice (i.e., lack of fetal T cell development, reduced cellularity in the adult thymus, increased in DN4 and CD4 single-positive thymocyte populations, reduced γδ T cells, increased Th1 and impaired Th2 differentiation, T cell hyperproliferation, and T cell transformation; see Refs. 14, 22, 25). Thus, Helios is clearly not required for Ikaros-dependent function in T cells and is not sufficient to sustain T cell development alone. Note, however, that Helios may play a unique role in more specialized T cell functions or subsets that were not studied here. The present mouse line thus provides an important tool to further explore the function of Helios in T cells.

The lack of apparent T cell phenotype in Helios-null mice suggests that other Ikaros family members may compensate for Helios in T cells. This is consistent with previous reports suggesting that Ikaros and Helios bind similar target sequences and belong to similar macromolecular complexes (5, 30). If so, then Ikaros appears to be the dominant family member in the /H9251/H9252 T cell lineage, while Helios may function in a redundant manner in these cells. It would therefore be interesting to study T cell development in animals deficient for both Ikaros and Helios. Our efforts to generate

FIGURE 6. Mature Treg cell function in Helios /H11002 mice. A, Purified WT and Helios /H11002 CD4+CD25+CD44+ Treg cells from spleen and lymph nodes were stimulated for 3 days with anti-CD3 and APCs, in the absence or presence of IL-2. Th cells (CD4+CD25+CD44+) were used as positive controls. CD4+CFSE+ cells were analyzed for CD25 expression and CFSE intensity. Numbers indicate percentages of cells in each gate. Results are representative of two independent experiments performed in triplicate. B, CFSE-stained responder Th cells (CD4+CD25+) were cultured for 3 days with anti-CD3 and APCs. WT and Helios /H11002 Treg cells (CD4+CD25+) were added at the indicated ratios. Th cells cultured with APCs in the absence of anti-CD3 are shown as the no stimuli control. Proliferation of Th cells is shown as CFSE loss in the CD4+CFSE–gated population. Numbers indicate percentages of cells in each gate. WT and Helios /H11002 Treg cells suppressed proliferation in a similar manner regardless of whether the Th cells and APCs were of WT or Helios /H11002 origin. Results are representative of two independent experiments performed in triplicate.

FIGURE 7. Expression of Ikaros, Aiolos, and Eos in Helios /H11002 cells. A, RNA from WT and Helios /H11002 thymocytes was analyzed for Aiolos, Ikaros, Eos, and truncated Helios transcripts by real-time RT-PCR. Expression levels were normalized to HPRT. Mean expression levels ± SD are presented, as calculated from three independent experiments. B, Nuclear extracts from WT and Helios /H11002 thymocytes were analyzed by Western blot using Helios, Aiolos, and Ikaros-specific Abs. TATA-box binding protein (TBP) was analyzed as a loading control. Results are representative of three independent experiments.
double-mutant mice have so far been unsuccessful, due to the extremely high mortality rate of these mice.

Recent studies have shown that Helios is highly expressed at the mRNA level in Treg cells compared with conventional CD4+ T cells (>10-fold) (31–33). Interestingly, Helios is expressed early in the Treg cell lineage and is not a downstream target of Foxp3 (45). Although the answer to this question will require the comparison of gene expression profiles between WT and Helios−/− Treg cells, the normal numbers and biological properties observed in the mutant Treg cells clearly indicate that Helios is not a master regulator of this lineage. However, a full dissection of Helios activity will require a better understanding of the functional redundancies among Ikaros family members, as well as the development of genetic models where Helios can be studied in combination with Ikaros and/or Eos deficiencies.

Interestingly, Helios deficiency does not alter the proliferative response of CD4+ and CD8+ T cells to TCR stimulation. This result was not anticipated, as overexpression of full-length Helios inhibits T cell proliferation in response to anti-CD3 stimulation (28), and the proliferative response of lymphocytes to Ag receptor-derived signals is known to be exquisitely sensitive to Ikaros family members. Indeed, loss of Ikaros or Aiolos leads to hyperproliferation in activated B and T cells (14, 15, 18, 26, 28). Our observations that Helios does not participate in this process may reflect a low relative abundance of Helios proteins compared with Ikaros and Aiolos in T cells, or a specific network of genes controlled by Ikaros or Aiolos, but not Helios.

Finally, several studies have linked the appearance of dominant-negative Helios to T cell transformation in both humans and mice. Our work does not support a prominent role for Helios as a primary tumor suppressor in T cells, although we cannot exclude the possibility that loss of Helios might cooperate with other oncogenic events to promote leukemogenesis. As Ikaros deficiency is consistently associated with T-acute lymphoblastic leukemia development in mouse models, we propose that T cell transformation occurs in animals (and perhaps isolated human cases) expressing ΔN Helios because these short isoforms bind and inhibit the activity of functional Ikaros proteins, and not functional Helios.

Acknowledgments

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