Published in final edited form as:
Leuk Res. 2010 April ; 34(4): 416–417. doi:10.1016/j.leukres.2009.10.010.

Tumor Suppression in T Cell Leukemia – The Role of Ikaros

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The Ikaros gene encodes a DNA-binding protein that belongs to the C2H2 zinc finger gene family [1]. Early biological experiments using mutant mouse models demonstrated that a lack of Ikaros expression in mice results in the absence of B, NK and dendritic cells, with a decreased number of T cells [2]. These experiments established Ikaros as a master regulator of lymphoid development. Subsequent analysis of Ikaros-deficient mice revealed that the absence of Ikaros expression, or the presence of dominant-negative Ikaros mutants, leads to the development of T cell leukemia in mice. This suggested that Ikaros acts as a tumor suppressor [3]. The identification of Ikaros as a master-regulator of lymphoid development and a tumor suppressor provided a novel model to study the development of leukemia, as well as tumor suppression, and raised hopes that Ikaros function might be important for tumor suppression in human leukemia. Ikaros was confirmed as a true tumor suppressor in the mouse by experiments in which the introduction of wild-type Ikaros into an Ikaros-deficient early-T leukemia cell line induced growth arrest along with T cell differentiation [4]. Ikaros has been shown to directly inhibit expression of the Notch target genes Hes-1 [5] and Deltex [6] suggesting that one possible mechanism for Ikaros-mediated suppression of leukemia involves inhibition of the Notch signaling pathway.

Cellular and biochemical experiments provided insight into the molecular mechanisms of Ikaros function. Ikaros DNA-binding ability and pericentromeric localization were shown to be essential for its function [7,8]. It was hypothesized that Ikaros binds the regulatory regions of its target genes resulting in their recruitment into pericentromeric heterochromatin leading to their activation or repression via chromatin remodeling [8]. A number of subsequent studies have provided evidence that essential Ikaros functions—DNA binding, pericentromeric localization, and regulation of gene expression, as well as the ability of Ikaros to suppress cellular proliferation, are regulated by posttranslational modifications and interaction with other proteins.

The ability of Ikaros to bind DNA is cell cycle-specific—hyperphosphorylation of specific amino acids at linkers that connect the DNA-binding zinc fingers, inhibits the ability of Ikaros

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Contribution of Authors Both authors contributed to developing an outline for the content of the manuscript, developing the initial draft of the manuscript, critically revising the intellectual content of the manuscript, and preparing the manuscript for submission. Both authors approve of the final draft for publication.

Conflict of interest The authors have no conflict of interest to declare.

to bind DNA during mitosis [9]. Ikaros is a substrate for CK2 kinase, and CK2 kinase phosphorylates Ikaros at multiple sites. CK2-mediated hyperphosphorylation of Ikaros results in the inability of Ikaros to control G1-S cell cycle progression [10], as well as a decrease in DNA-binding affinity and a loss of its pericentromeric localization [11]. Recent evidence indicates that CK2-mediated phosphorylation also leads to increased degradation of Ikaros via the ubiquitin pathway [12]. These studies suggest that phosphorylation of Ikaros by CK2 kinase may be an important mechanism for inactivating Ikaros tumor suppressor activity in lymphocytes. This hypothesis is in accord with studies of transgenic mice showing that increased expression of CK2 kinase results in T cell leukemia of a type similar to that observed in Ikaros-deficient mice [13].

Sumoylation of Ikaros has been identified as a mechanism for regulating its association with the repressive histone deacetylase (NuRD) complex, which may affect Ikaros function as a transcriptional repressor or activator [14]. Ikaros can also form a complex with the CALM/AF10 fusion protein and interaction with this protein complex results in the loss of Ikaros pericentromeric localization [15]. Increased expression of small dominant negative Ikaros isoforms has been associated with the development of acute myelogenous leukemia (AML) [16].

Taken together these studies suggest that Ikaros function as a tumor suppressor can be regulated by two mechanisms: 1) **genetic inactivation** due to deletion or mutation that results in absent or nonfunctional Ikaros protein and/or by 2) **functional inactivation** where the Ikaros gene sequence remains intact but the protein is inactivated due to posttranslational modifications or interaction with various proteins (e.g. CALM/AF10 or small dominant negative Ikaros isoforms) that result in the loss of Ikaros tumor suppressor function.

Extensive analysis of the Ikaros gene locus has provided conclusive evidence for the genetic inactivation of Ikaros in human B cell leukemia [17–19]. Approximately 30% of pediatric B cell acute lymphoblastic leukemia (ALL) cases showed genetic inactivation of Ikaros. The majority of these leukemia cells harbored an Ikaros deletion, although a small number of leukemias exhibited mutations that were either nonsense or missense, or resulted in inactive protein [19]. Deletion of Ikaros was particularly prominent (~80%) in BCR-ABL1 ALL—a subset that is associated with poor prognosis [18]. In B-cell ALL without the BCR-ABL1 translocation, deletion of Ikaros was associated with poor prognosis, which underscored the role of Ikaros in tumor suppression [19]. These data strongly suggest that deletion is a frequent mode of genetic inactivation in human B cell ALL.

In this issue of Leukemia Research, Marcais et al. provide sequence analysis, along with RT-PCR, Western blot and CGH array analysis of Ikaros in 25 cases of human T cell ALL [20]. This detailed analysis identified 1 patient (4% of total cases) with an Ikaros deletion that also resulted in altered subcellular localization of the Ikaros protein. No point mutations (except for one silent mutation) were detected in T cell ALL samples. Furthermore, the high expression of Hes1, an indication of increased Notch pathway activity, observed in several patients, did not correlate with the presence of the Ikaros deletion [20]. These findings provide an important insight into pathogenesis of T cell ALL. While genetic inactivation of Ikaros is a frequent event associated with human B ALL, that does not seem to be the case in T ALL. This study by Marcais et al. demonstrates that Ikaros deletion, albeit a rare event (4% of cases), correlates with changes in Ikaros function (pericentromeric localization) and with T cell ALL, implying that Ikaros has a tumor suppressor role in this type of leukemia.

Thus, the work by Marcais et al. provides important direction for future studies of Ikaros in T cell ALL and other leukemias. Data from murine biological models, together with the identification of Ikaros in ~4% patients with T-cell ALL, strongly suggest that Ikaros acts as

a tumor suppressor in T cell ALL. The data presented by Marcais et al. imply that mechanisms other than deletion and/or point mutation are likely to play a role in the regulation of Ikaros function. This study highlights the potential role for functional inactivation of Ikaros as a contributor to T cell ALL and the importance of studies that compare Ikaros function in T-ALL cells and their normal physiological counterparts. Further studies that examine the role of ubiquitination, phosphorylation, protein interaction with CALM/AF10 and other Ikaros isoforms, as they relate to Ikaros function in T cell ALL, should provide a better understanding of the pathogenesis of T cell ALL and other leukemias.

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