Ikaros isoforms: The saga continues

Zhanjun Li, Laura A Perez-Casellas, Aleksandar Savic, Chunhua Song, Sinisa Dovat

Zhanjun Li, Chunhua Song, Sinisa Dovat, Department of Pediatrics, Pennsylvania State University, College of Medicine, H085, Division of Pediatric Hematology/Oncology, Hershey, PA 17033-0850, United States
Laura A Perez-Casellas, Department of Pediatrics, University of Wisconsin-Madison, Madison, WI 53792, United States
Aleksandar Savic, Clinic of Hematology, Clinical Center of Vojvodina, Faculty of Medicine, University in Novi Sad, Novi Sad 21000, Serbia

Author contributions: Song C, Li Z and Savic A were responsible for the bibliographic research; Perez-Casellas LA and Song C were responsible for critically reviewing and revising the manuscript; Dovat S prepared the initial draft of the manuscript and edited it for final revisions.

Supported by (in part) An R01 HL095120 grant, a St. Baldrick’s Foundation Career Development Award, the Four Diamonds Fund of the Pennsylvania State University, College of Medicine, and the John Wawrynovic Leukemia Research Scholar Endowment (SD)

Correspondence to: Sinisa Dovat, MD, PhD, Associate Professor of Pediatrics, Four Diamonds Endowed Chair, of Medicine, H085, Division of Pediatric Hematology/Oncology, 500 University Drive, PO Box 850, Hershey, PA 17033-0850, United States. sdovat@hmc.psu.edu

Telephone: +1-717-5310003-280286 Fax: +1-717-5314789
Received: March 22, 2011 Revised: May 5, 2011
Accepted: May 12, 2011
Published online: June 26, 2011

Abstract

Through alternate splicing, the Ikaros gene produces multiple proteins. Ikaros is essential for normal hematopoiesis and possesses tumor suppressor activity. Ikaros isoforms interact to form dimers and potentially multimeric complexes. Diverse Ikaros complexes produced by the presence of different Ikaros isoforms are hypothesized to confer distinct functions. Small dominant-negative Ikaros isoforms have been shown to inhibit the tumor suppressor activity of full-length Ikaros. Here, we describe how Ikaros activity is regulated by the coordinated expression of the largest Ikaros isoforms IK-1 and IK-H. Although IK-1 is described as full-length Ikaros, IK-H is the longest Ikaros isoform, IK-H, which includes residues coded by exon 3B (60 bp that lie between exons 3 and 4), is abundant in human but not murine hematopoietic cells. Specific residues that lie within the 20 amino acids encoded by exon 3B give IK-H DNA-binding characteristics that are distinct from those of IK-1. Moreover, IK-H can potentiate or inhibit the ability of IK-1 to bind DNA. IK-H binds to the regulatory regions of genes that are upregulated by Ikaros, but not genes that are repressed by Ikaros. Although IK-1 localizes to pericentromeric heterochromatin, IK-H can be found in both pericentromeric and non-pericentromeric locations. Anti-silencing activity of gamma satellite DNA has been shown to depend on the binding of IK-H, but not other Ikaros isoforms. The unique features of IK-H, its influence on Ikaros activity, and the lack of IK-H expression in mice suggest that Ikaros function in humans may be more complex and possibly distinct from that in mice.

© 2011 Baishideng. All rights reserved.

Key words: Ikaros; Chromatin; Pericentromeric; Transcription; IK-H; Leukemia; γ satellite

Peer reviewers: Carlo Ventura, MD, PhD, Full Professor of Molecular Biology, Chief, Laboratory of Molecular Biology and Stem Cell Engineering-National Institute of Biostructures and Biosystems, University of Bologna, S. Orsola - Malpighi Hospital, Cardiovascular Department, Pavilion 21 Via Massarenti 9 Bologna 40138, Italy; Guillermo Montoya, Professor, Structural Biology and Biocomputing, Spanish National Cancer Research Centre, Melchor Fdez. Almagro 3, 28029 Madrid, Spain; Koji Hisatake, Professor, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan

Li Z, Perez-Casellas LA, Savic A, Song C, Dovat S. Ikaros isoforms: The saga continues. World J Biol Chem 2011 June 26; 2(6): 140-145 Available from: URL: http://www.wjgnet.com/1949-8454/full/v2/i6/140.htm DOI: http://dx.doi.org/10.4331/wjbc.v2.i6.140

IKAROS ISOFORM EXPRESSION AND IKAROS ACTIVITY

An important factor in the functional diversity of indi-
vidual genes is their ability to encode different proteins. Many genes produce multiple proteins that can have di-
similar functions through alternate splicing. This process
is responsible for the generation of complex structural
and regulatory networks that control normal cellular
function. Determination of the functional similarities and
differences of individual isoforms is essential for under-
standing the role of a gene in a particular process.

The Ikaros gene encodes a zinc finger protein that is
essential for normal hematopoiesis and that acts as a tu-
mor suppressor[1-3]. The Ikaros protein binds DNA at the
upstream regulatory elements of its target genes and regu-
lates their transcription via chromatin remodeling. Soon
after discovery of the Ikaros gene, it was determined that
it encodes multiple isoforms via alternate splicing[4,5]. In
mice, the full-length Ikaros isoform comprises three do-
mains: (1) a DNA-binding domain (at the N-terminal end
of the protein) that consists of four C2H2 Kruppel-like
zinc finger motifs; (2) a protein-interaction domain (at the
C-terminal end of the protein) that consists of two zinc
finger motifs with an unusual structure that is most simi-
lar to those described in the hunchback gene in Drosophila,
and (3) an activation domain that is a loosely defined
domain that is located between the DNA-binding and
protein-interaction domains[6]. Every Ikaros isoform de-
scribed thus far contains the protein-interaction domain,
but they differ in the presence of the DNA-binding do-
mains, or other regions of the gene. Ikaros associates in vivo
with its various isoforms, as well as with other Ikaros family
members that contain a protein-interaction domain similar to that of Ikaros[7]. Ikaros binds DNA as a dimer
and possibly as a multimer, therefore, it has been hypo-
thesized that diverse complexes that comprise full-length
Ikaros together with different types of isoforms could
have distinct functions[8,9]. The ability of the different
Ikaros isoforms to interact with each other and with other
Ikaros family members[7,10,11] creates many possible com-
binations of proteins with the potential for diverse func-
tions: four major possibilities are summarized in Figure 1.

Studies of mice with mutations in the Ikaros gene, to-
gether with biochemical experiments, have revealed that
small Ikaros isoforms that contain a protein-interaction
domain and that lack a DNA-binding domain exert a
dominant-negative (DN) effect by inhibiting the activity
of the full-length Ikaros protein[9]. This is demonstrated in
Ikaros knockout mice that express small DN isoforms.
These mice have a more severe phenotype when com-
pared to “true null” Ikaros knockout mice[2,12,13]. Over-
expression of DN isoforms disrupts normal hematopoiesis and
has been shown to inhibit normal Ikaros function in num-
erous in vitro and in vivo systems[14,15]. In biochemical
experiments, overexpression of DN isoforms inhibits
DNA-binding of full-length Ikaros. High expression of
DN Ikaros isoforms has been associated with hematopoi-
etic malignancies in humans and mice, as well as with
pituitary gland tumors[15,16-20]. It has been hypothesized
that this is most likely due to inhibition of the tumor
suppressor function of Ikaros and possibly other family
members. Thus, the first established functional relation-

ship between Ikaros isoforms was relatively clear cut-
full-length Ikaros is functional and a tumor suppressor,
whereas small DN isoforms are inhibitory and pro-onco-
genic. A mechanistic explanation for this involved inhibi-
tion of the DNA-binding ability of the full-length Ikaros
isoform by small DN ones. This involved the possibility
depicted in Figure 1C.

Deciphering the functional significance of other large
Ikaros isoforms has proved to be more complicated.
One particular problem is the abundance of endogenous
full-length Ikaros isoforms in most hematopoietic cells,
which makes it difficult to study the function of indi-
vidual large Ikaros isoforms in vivo. However, over time,
data have emerged to reveal the potential functional sig-
nificance of other Ikaros isoforms. Ikaros-2 (Ikaros-V
in the nomenclature of the Smale group), which lacks
the first N-terminal DNA-binding zinc finger, has been
shown to produce a footprint distinct from that of 1K-1
in DNase protection assays[9], although the functional
significance of this has not been studied. A report by
Payne et al[21] has revealed that the Ikaros-X isoform has a
unique expression pattern compared to other large Ikaros
isoforms, and has suggested that this isoform plays a role
in myeloid differentiation. This study was limited to ex-
pression analysis and did not provide detailed functional
analysis of the Ikaros-X isoform.

Most functional studies of Ikaros isoforms have been
performed using murine transcripts and proteins. An early murine study has detected the presence of
Ikaros protein and cDNA that includes coding sequence
that lies between currently designated Ikaros exons 3 and 4[9]. Human Ikaros gene transcripts that incorpo-
rate the human homolog of this additional exon (which we
designate exon 3B) have been subsequently detected in
leukemia samples[9]. Studies of Ikaros protein expression
in normal human hematopoietic cells have identified sev-
eral Ikaros splice forms that include exon 3B, including
one larger in size than any of the previously described
murine Ikaros isoforms[21,22]. Comparisons of Ikaros
expression in mice and humans have shown that strong
protein level expression of this isoform occurs only in
human cells[21,22]. For this reason, the largest Ikaros splice
variant is termed Ikaros-H (H for human). Due to the
high sequence homology between the murine and hu-
man Ikaros genes, the activity of individual isoforms has
been assumed to be very similar. The high level protein
expression of Ikaros-H in human, but not murine cells is
an intriguing observation that suggests that the regulation
of Ikaros activity in human cells might be more complex
than in mouse cells. Other than Ikaros-1 (IK-1, and in the
nomenclature of the Smale group, Ikaros-VI), Ikaros-H
is the subject of the most extensive functional
studies to date. The following summarizes the functional
characteristics of the Ikaros-H isoform.

**EXPRESSION PATTERN AND DNA BINDING AFFINITY OF IK-H**

The Ikaros-H isoform (IK-H, also designated IK-1+ in
alternate nomenclature\cite{22} contains the sequence of the “full-length” Ikaros isoform (IK-1) plus an additional 20 amino acids N-terminal to the DNA-binding zinc fingers (Exon 3B in Figure 2A). In mice, the most abundant Ikaros isoforms in primary T and B lineage cells and in lymphoid leukemia cells are IK-1 and IK-2, while IK-H is essentially absent\cite{21,23}. In contrast, in humans, IK-1, IK-2 and IK-H are expressed at comparable levels in primary leukemia cells and in lymphoid cell lines. IK-H is also abundant in primary human T and B cells\cite{21}. Thus, IK-H exhibits a species-specific expression pattern.

Ikaros contains four C2H2 Kruppel-like zinc finger motifs at its N-terminal end (exons 4–6) that directly interact with DNA. Zinc fingers 2 and 3 are essential for DNA binding of Ikaros protein, although other zinc fingers probably contribute to DNA-binding specificity of Ikaros\cite{23}. Ikaros isoforms bind to the Ikaros consensus sequence TGGGAA/T with the core sequence comprising GGGA\cite{24}. DNA binding analysis has revealed that the two largest human isoforms, IK-1 and IK-H, have different DNA-binding affinities\cite{23}. Both isoforms can bind to the DNA sequences that contain the TGGGAA/T consensus site, as well as to high-affinity sites where two core consensus GGGA sequences are present within 40 bp of each other. However, only IK-1 is able to bind efficiently to DNA sequences that contain a single core sequence, GGGA; the absence of the second GGGA consensus sequence abolishes DNA binding by IK-H. This result was surprising and suggests that the presence of the 20 amino acids that are located upstream from the DNA-binding zinc fingers in IK-H have an inhibitory effect on DNA binding of Ikaros protein. Substitution mutational analysis\cite{22} has identified three specific amino acids within the N region that are responsible for the differences in DNA binding of IK-H and IK-1. This suggests that it is not the presence of the additional 20 amino acids N-terminal of the DNA-binding zinc fingers that affects the ability of the Ikaros protein to bind DNA, but rather interaction of specific residues within the 20-amino-acid region with unknown proteins.

**IK-H REGULATES DNA BINDING OF OTHER IKAROS ISOFORMS**

Evidence that the two largest human Ikaros isoforms have different DNA-binding affinities has led us to speculate that the DNA-binding affinity of Ikaros proteins for a specific DNA sequence could depend on the relative expression levels of IK-1 and IK-H isoforms. We hypothesize that IK-H could either synergize with IK-1 or act to inhibit its DNA-binding, depending on its unique affinity for a particular DNA sequence. This hypothesis has been tested in vitro by mixing recombinant IK-1 and IK-H isoforms and comparing the DNA-binding affinity of the IK-1/IK-H mixture with the same quantity of IK-1 protein. IK-1 and IK-H act synergistically to bind DNA that contains two Ikaros binding sites. However, the IK-H isoform inhibits the binding of IK-1 to DNA that contains a single Ikaros binding site\cite{23}. Thus, IK-H can either potentiate or inhibit the DNA binding of the IK-1 isoform in vitro.

It has been demonstrated that Ikaros binds DNA in vivo as a dimer and possibly as a multimeric complex\cite{23}. To test whether the interaction of IK-1 and IK-H regulates the DNA-binding affinity of Ikaros in vivo, the ability of Ikaros to bind DNA has been compared using electrophoretic mobility shift assay and chromatin immunoprecipitation (ChIP) to assess cells that expressed only IK-1, only IK-H, or both isoforms. Expression of IK-H selectively affects the DNA-binding of Ikaros proteins - DNA-binding to the DNA sequences that contain a single Ikaros binding site is reduced, but binding to DNA with two Ikaros consensus binding sites is strong. These experiments were performed in activated T cells, thus confirming the physiological relevance of IK-H interactions with IK-1. These results demonstrate that IK-H does not function as a typical DN isoform (as described for the small Ikaros isoforms that lack DNA binding zinc fingers), but rather as a unique control mechanism that determines DNA-binding specificity of Ikaros proteins in vivo. This provides confirmation that Ikaros binds DNA in vivo as a dimer or multimer, and for the first time has demonstrated the functional significance of the coexpression of different large Ikaros isoforms: to provide precise control of the DNA-binding specificity of Ikaros in cells.

**SUBCELLULAR LOCALIZATION OF THE LARGEST IKAROS ISOFORMS**

The condensed chromatin that flanks centromeres is known as pericentromeric heterochromatin (PC-HC). Genes localized to PC-HC are generally transcriptionally
inactive. The subcellular localization of Ikaros to PC-HC has been shown to correlate directly with its DNA-binding affinity towards the repetitive sequences that are part of PC-HC. Confocal microscopy has revealed that IK-1 and IK-H exhibit distinct subcellular localization. The IK-1 isoform displays the typical punctate distribution pattern consistent with PC-HC localization. In contrast, IK-H exhibits dual - non-centromeric and centromeric - localization. The non-centromeric localization of IK-H is not in the form of the diffuse nuclear staining that is typically observed in other transcriptional factors, but rather localization to other specific nuclear structures. More intriguing is that the non-centromeric localization of IK-H is observed in hematopoietic cells, but not when this isoform is transduced into fibroblasts. When transduced in murine hematopoietic cells, IK-H retains the same localization pattern as in human cells. This suggests that the distinct localization pattern of IK-H is tissue-specific and that it is a result of the unique properties of the IK-H protein, and not due to the differences in heterochromatin between different species. This also provides evidence that IK-1 exists in complexes that do not contain the IK-1 isoform and thus, it may have its own unique cellular function. This provides the first evidence to suggest that different Ikaros isoforms may function in distinct subcellular regions.

ROLE OF IK-H IN GENE ACTIVATION AND CHROMATIN REMODELING

It has been demonstrated that Ikaros can act both as a positive and a negative regulator of gene expression. This is likely related to the ability of Ikaros to associate with the SWI/SNF activator complex or with the histone deacetylase repressor complex, NuRD. The evidence that Ikaros can both activate and repress expression of its target genes by recruiting them to PC-HC suggests a role for chromatin remodeling in this process. The molecular mechanisms that determine whether Ikaros functions as an activator or repressor of transcription are unknown.

Experiments that have tested the DNA-binding specificities of IK-1 and IK-H isoforms to regulatory sequences of known Ikaros target genes have revealed differential binding of these isoforms to regulatory elements of repressed vs activated Ikaros targets. Binding of both IK-H and IK-1 to the upstream regulatory elements of IKCa1, Granzyme B, STAT4, and FAAH has been demonstrated by ChIP and electromobility supershift assay. In contrast, IK-1 and other Ikaros isoforms, but not IK-H, bind the upstream regulatory element of the VPAC-1 receptor gene. In vivo, Ikaros proteins downregulate transcription of the VPAC1 receptor gene, while positively regulating expression of IKCa1, Granzyme B, STAT4, and FAAH. The observation that the IK-H isoform binds to the regulatory region of genes upregulated by Ikaros, but not to the regulatory region of genes repressed by Ikaros has led to the intriguing hypothesis that the IK-H isoform acts as an activator, while other Ikaros isoforms act as transcriptional repressors of Ikaros target genes. According to this hypothesis, IK-H would have the opposite function of other Ikaros isoforms in regulating transcription. The mechanism by which Ikaros would activate its target genes upon their recruitment to PC-HC has not been resolved.

A detailed analysis of the function of human gamma-satellite DNA repeats reveals that gamma-satellite DNA has anti-silencing activity. ChIP analysis has documented binding of both IK-H and IK-1 to the gamma-satellite DNA. Mutational analysis reveals that the anti-silencing
activity of gamma-satellite DNA is directly dependent on the presence of two consensus Ikaros binding sites that are in close proximity (bipartite motif), and on the binding of IK-H. The mutation of one Ikaros consensus binding site that abolishes the binding of IK-H, but not binding of other Ikaros isoforms, leads to the loss of the anti-silencing activity of gamma-satellite DNA[34]. These results clearly showed that: (1) human gamma-satellite DNA allows a transcriptionally permissive chromatin conformation, thus it can have an anti-silencing function; and (2) binding of the IK-H Ikaros isoform (but not other Ikaros isoforms) is essential for the anti-silencing activity of gamma-satellite DNA. This sheds light on the molecular mechanisms by which Ikaros can both activate or repress its target genes via recruitment to PC-RC and establishes distinct (and possibly opposing) roles for IK-H and IK-1 isoforms in the regulation of gene expression and chromatin remodeling. The current (simplified) model of transcriptional regulation by IK-H and IK-1 is outlined in Figure 2B.

CONCLUSION

In summary, the Ikaros gene encodes a large number of different proteins via alternate splicing, Ikaros-encoded proteins form dimers and multimers that regulate gene expression and chromatin remodeling. The presented analysis emphasizes that the function of Ikaros complexes depends on the presence of particular Ikaros isoforms, and that individual isoforms likely have unique functions. High expression of the IK-H isoform in human lymphoid cells, but not their murine counterparts, suggests that the regulatory function of Ikaros in humans is more complex and possibly distinct from that in mice.

The discovery of functional differences between IK-1 and IK-H isoforms raises many questions that need to be resolved. Future research will be directed to achieve the following goals: (1) dissection of the DNA-binding specificity of IK-1 and IK-H using global functional genomic approaches; and (2) identification of additional genes that are regulated by Ikaros and a correlation of the binding of IK-1 and IK-H to upstream regulatory regions with changes in target gene expression. Further analysis of the function of Ikaros proteins is essential for understanding their role in normal human hematopoiesis and their tumor suppressor function in human leukemia.

REFERENCES

1. Georgopoulos K, Bigby M, Wang JH, Molnar A, Wu P, Winandy S, Sharpe A. The Ikaros gene is required for the development of all lymphoid lineages. Cell 1994; 79: 143-156
2. Georgopoulos K, Moore DD, Derfler B. Ikaros, an early lymphoid-specific transcription factor and a putative mediator for T cell commitment. Science 1992; 258: 808-812
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: 289-299
4. Molnár A, Georgopoulos K. The Ikaros gene encodes a family of functionally diverse zinc finger DNA-binding proteins.
5. Sun L, Liu A, Georgopoulos K. Zinc finger-mediated protein interactions modulate Ikaros activity, a molecular control of lymphocyte development. EMBO J 1996; 15: 5338-5349
6. Hahn K, Ernst P, Lo K, Kim GS, Turck C, Smale ST. The lymphoid transcription factor Lyf-1 is encoded by specific, alternatively spliced mRNAs derived from the Ikaros gene. Mol Cell Biol 1994; 14: 7111-7123
7. Hahn K, Cobb BS, McCarty AS, Brown KE, Klug CA, Lee R, Akashi K, Weissman IL, Fisher AG, Smale ST. Helios, a T cell-restricted Ikaros family member that quantitatively associates with Ikaros at centromeric heterochromatin. Genes Dev 1998; 12: 782-796
8. Morgan B, Sun L, Avitahl N, Andrikopoulos K, Ikeda T, Gonzales E, Wu P, Neben S, Georgopoulos K. Aiolos, a lymphoid restricted transcription factor that interacts with Ikaros to regulate lymphocyte differentiation. EMBO J 1997; 16: 2004-2013
9. Klug CA, Morrison SJ, Masek M, Hahn K, Smale ST, Weissman IL. Hematopoietic stem cells and lymphoid progenitors express different Ikaros isoforms, and Ikaros is localized to heterochromatin in immature lymphocytes. Proc Natl Acad Sci USA 1998; 95: 657-662
10. Dovat S, Montecino-Rodriguez E, Schuman V, Teitell MA, Dorschkind K, Smale ST. Transgenic expression of Helios in B lineage cells alters B cell properties and promotes lymphomagenesis. J Immunol 2005; 175: 3508-3515
11. Alinikula J, Kohonen P, Nera KP, Lassila O. Concerted action of Helios and Ikaros controls the expression of the invositol 5-phosphatase SHIP. Eur J Immunol 2010; 40: 2599-2607
12. Wu L, Nichogiannopoulos A, Shortman K, Georgopoulos K. Cell-autonomous defects in dendritic cell populations of Ikaros mutant mice point to a developmental relationship with the lymphoid lineage. Immunity 1997; 7: 483-492
13. Wang JH, Nichogiannopoulos A, Wu L, Sun L, Sharpe AH, Bigby M, Georgopoulos K. Selective defects in the development of the fetal and adult lymphoid system in mice with an Ikaros null mutation. Immunity 1996; 5: 537-549
14. Tonnelle C, Bardin F, Maroc C, Imbert AM, Campa F, Dal-loul A, Schmitt C, Chabannon C. Forced expression of the Ikaros 6 isoform in human placental blood CD34(+) cells impairs their ability to differentiate toward the B-lymphoid lineage. Blood 2001; 98: 2673-2680
15. Ezzat S, Yu S, Asa SL. Ikaros isoforms in human pituitary tumors: distinct localization, histone acetylation, and activation of the 5’ fibroblast growth factor receptor-4 promoter. Am J Pathol 2003; 163: 1177-1184
16. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, Girtman K, Mathew S, Ma J, Pounds SB, Su X, Pui CH, Relling MV, Evans WE, Shurtleff SA, Downing JR. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 2007; 446: 758-764
17. Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J, White D, Hughes TP, Le Beau MM, Pui CH, Relling MV, Shurtleff SA, Downing JR, BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. Nature 2008; 453: 110-114
18. Kuiper RP, Schoenmakers EF, van Reijmersdal SV, Hehir-Kwa JY, van Kessel AG, van Leeuwen FN, Hoogerbrugge PM. High-resolution genomic profiling of childhood ALL reveals novel recurrent genetic lesions affecting pathways involved in lymphocyte differentiation and cell cycle progression. Leukemia 2007; 21: 1258-1266
19. Sun L, Heerema N, Crotty L, Wu X, Navara C, Vassilev A, Sensel M, Reaman GH, Uckun FM. Expression of dominant-negative and mutant isoforms of the antileukemic transcription factor Ikaros in infant acute lymphoblastic leukemia. Proc Natl Acad Sci USA 1999; 96: 680-685
20. Yagi T, Hibi S, Takanushi M, Kano G, Tabata Y, Imamura T, Inaba T, Morimoto A, Todo S, Imashuku S. High frequency
of Ikaros isoform 6 expression in acute myelomonocytic and monocytic leukemias: implications for up-regulation of the antiapoptotic protein Bcl-XL in leukemogenesis. Blood 2002; 99: 1350-1355

21 Payne KJ, Huang G, Sahakian E, Zhu JY, Barteneva NS, Barsky LW, Payne MA, Crooks GM. Ikaros isoform x is selectively expressed in myeloid differentiation. J Immunol 2003; 170: 3091-3098

22 Payne KJ, Nicolas JH, Zhu JY, Barsky LW, Crooks GM. Cutting edge: predominant expression of a novel Ikaros isoform in normal human hemopoiesis. J Immunol 2003; 170: 3091-3098

23 Ronni T, Payne KJ, Ho S, Bradley MN, Dorsam G, Dovat S. Human Ikaros function in activated T cells is regulated by coordinated expression of its largest isoforms. J Biol Chem 2007; 282: 2538-2547

24 Cobb BS, Morales-Alce day S, Kleiger G, Brown KE, Fisher AG, Smale ST. Targeting of Ikaros to pericentromeric heterochromatin by direct DNA binding. Genes Dev 2000; 14: 2146-2160

25 Trinh LA, Ferrini R, Cobb BS, Weinmann AS, Hahn K, Ernst P, Garraway LP, Merkenschlager M, Smale ST. Down-regulation of TDT transcription in CD4+CD8+ thymocytes by Ikaros proteins in direct competition with an Ets activator. Genes Dev 2001; 15: 1817-1832

26 Koipally J, Renold A, Kim J, Georgopoulos K. Repression by Ikaros and Aiolos is mediated through histone deacetylase complexes. EMBO J 1999; 18: 3090-3100

27 Kim J, Sif S, Jones B, Jackson A, Koipally J, Heller E, Winandy S, Viel A, Sawyer A, Ikeda T, Kingston R, Georgopoulos K. Ikaros DNA-binding proteins direct formation of chromatin remodeling complexes in lymphocytes. Immunity 1999; 10: 345-355

28 O’Neill DW, Schötz SS, Lopez RA, Castle M, Rabinowitz L, Shor E, Krawchuk D, Golli MG, Renz M, Seelig HP, Han S, Seong RH, Park SD, Agalioti T, Munshi N, Thamso D, Erdjument-Bromage H, Tempst P, Bank A. An ikaros-containing chromatin-remodeling complex in adult-type erythroid cells. Mol Cell Biol 2000; 20: 7572-7582

29 Koipally J, Heller EJ, Seavitt JR, Georgopoulos K. Unconventional potentiation of gene expression by Ikaros. J Biol Chem 2002; 277: 13007-13015

30 Dorsam G, Goetzl EJ. Vasoactive intestinal peptide receptor-1 (VPAC-1) is a novel gene target of the hemolymphopoietic transcription factor Ikaros. J Biol Chem 2002; 277: 13488-13493

31 Ghanshani S, Wulff H, Miller MJ, Rohm H, Neben A, Gutman GA, Cahalan MD, Chandy KG. Up-regulation of the IKCa1 potassium channel during T-cell activation. Molecular mechanism and functional consequences. J Biol Chem 2000; 275: 57137-57149

32 Wargnier A, Lafaurie C, Legros-Maida S, Bourge JF, Sigaux F, Sasportes M, Paul P. Down-regulation of human granzyme B expression by glucocorticoids: Desamethasone inhibits binding to the Ikaros and AP-1 regulatory elements of the granzyme B promoter. J Biol Chem 1998; 273: 35326-35331

33 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: 4470-4478

34 Kim JH, Ebersole T, Kouprina N, Noskov VN, Ohzeki J, Masumoto H, Dravinac B, Sullivan BA, Pavlicaek A, Dovat S, Pack SD, Kwon YW, Flanagan PT, Loukinov D, Lobanenkov V, Larionov V. Human gamma-satellite DNA maintains open chromatin structure and protects a transgene from epigenetic silencing. Genome Res 2009; 19: 533-544

S- Editor Cheng JX  L- Editor Kerr C  E- Editor Zheng XM