Dominant Negative Isoform of the Ikaros Gene in Patients with Adult B-Cell Acute Lymphoblastic Leukemia


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Abstract

Gene targeting studies in mice have shown that the transcription factor Ikaros plays an essential role in lymphoid development and as a tumor suppressor in B cells. We analyzed the expression levels of the Ikaros gene family, Ikaros and Aiolos, in human bone marrow samples from patients with adult acute lymphoblastic leukemia [ALL (n = 46; B-cell ALL = 41; T-cell ALL = 5)]. Overexpression of the dominant negative isoform of Ikaros gene Ik-6 was observed in 14 of 41 B-cell ALL patients by reverse transcription-PCR, and the results were confirmed by sequencing analysis and immunoblotting. None of the other dominant negative isoforms of the Ikaros gene were detected by reverse transcription-PCR analysis. Southern blotting analysis with PirI digestion revealed that those patients with the dominant negative isoform Ik-6 might have small mutations in the Ikaros locus. We did not detect any overexpression of dominant negative isoforms of Aiolos in adult ALL patients. These results suggest that Ikaros plays a key role in human B-cell malignancies through the dominant negative isoform Ik-6.

Introduction

The Ikaros gene has been shown in the murine model to be essential for the development of B lymphocytes and to function as a tumor suppressor in T cells, whereas the related gene Aiolos functions as a tumor suppressor in B cells. We analyzed the expression levels of the Ikaros gene family, Ikaros and Aiolos, in human bone marrow samples from patients with adult acute lymphoblastic leukemia [ALL (n = 46; B-cell ALL = 41; T-cell ALL = 5)]. Overexpression of the dominant negative isoform of Ikaros gene Ik-6 was observed in 14 of 41 B-cell ALL patients by reverse transcription-PCR, and the results were confirmed by sequencing analysis and immunoblotting. None of the other dominant negative isoforms of the Ikaros gene were detected by reverse transcription-PCR analysis. Southern blotting analysis with PirI digestion revealed that those patients with the dominant negative isoform Ik-6 might have small mutations in the Ikaros locus. We did not detect any overexpression of dominant negative isoforms of Aiolos in adult ALL patients. These results suggest that Ikaros plays a key role in human B-cell malignancies through the dominant negative isoform Ik-6.

Materials and Methods

RT-PCR Analysis. Bone marrow aspirates were obtained from patients after obtaining informed consent. Characteristics of adult B-ALL patients are shown in Table 1. All patients had a negative myeloperoxidase test. RT-PCR analysis was performed as described previously (11). The sequences of the primers used are as follows: (a) Ikaros sense, 5′-CACATAAACCTGAGACACATG-3′; (b) Ikaros antisense, 5′-AGGGCTTAGCTCAGTGGC-3′ (4); (c) Aiolos sense, 5′-CCCCGCACGAGCATGAGA-3′; and (d) Aiolos antisense, 5′-CCGAATTCCTCAGCAGC-3′ (8). Bone marrow samples from five T-ALL, six ALL (L3), and five adult T-cell leukemia patients were also examined. Major and minor BCR/ABL fusion transcripts were analyzed by RT-PCR (Table 1).

Sequencing Analysis. The PCR products were subcloned into the pCR2.1-TOPO vector using the TOPO TA Cloning Kit (Invitrogen, San Diego, CA).

Received 3/23/00; accepted 6/8/00.

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2 The abbreviations used are: T-ALL, T-cell acute lymphoblastic leukemia; ALL, acute lymphoblastic leukemia; B-ALL, B-cell acute lymphoblastic leukemia; RT-PCR, reverse transcription-PCR.
The sequencing analysis was performed using the ABI 373 DNA Sequencer (Perkin-Elmer, Foster City, CA).

**Immunoblotting.** Extraction of whole cell lysates and immunoblotting were performed as described previously (15). The membranes were incubated with anti-Ikaros antibody or anti-Sp1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

**Southern Blotting.** Southern blotting was performed as described previously (16). The human Ikaros cDNA used as a hybridization probe was labeled with [α-32P]dCTP using the High Prime DNA labeling kit (Boehringer Mannheim, Indianapolis, IN).

**Results.**

The expression levels of the Ikaros family genes, Ikaros (4) and Aiolos (8), in bone marrow samples from adult ALL patients (n = 46; B-ALL = 41; T-ALL = 5) were analyzed by performing RT-PCR (Fig. 1 and data not shown). The expression of the largest isoforms (Ik-1, Ik-2, and Ik-3) of the Ikaros gene was predominant, as described previously (4). However, in 14 of 41 patients with adult B-ALL, the dominant negative isoform Ik-6 was the predominant isoform expressed (Fig. 1 and Table 1; patients 1–4, 15–17, and 35–41). These results were confirmed by sequencing analysis (data not shown). In contrast to the previous reports (12–14), none of the other dominant negative isoforms (Ik-7 and Ik-8) were found in adult ALL patients, although Ik-4 is normally expressed at a low level. There was a trend that expression of the dominant negative isoform Ik-6 was correlated with expression of BCR/ABL fusion transcripts (Table 1), although it was not statistically significant (χ² test, P = 0.18). We found no dominant negative isoforms of the Ikaros gene in bone marrow samples from five T-ALL, six ALL (L3), and five adult T-cell leukemia patients (data not shown).

The Ikaros family member gene Aiolos was shown in mice to be important for regulated proliferation of B cells (7). We therefore determined whether altered levels of Aiolos expression correlated with incidence of B-ALL. Unlike Ikaros, which has several splice forms, Aiolos has only one splice form. In the patient samples examined, we detected only the reported isoform of Aiolos; no dominant negative isoforms were observed. However, Aiolos expression appeared to be decreased in several patients (Fig. 1; patients 1, 14, 15, 25, 32, 35, 37, and 38).

The overexpression of the dominant negative Ik-6 protein in bone marrow cells from adult ALL patients was confirmed by immunoblotting of whole cell lysates using an anti-Ikaros antibody. Whole cell lysate from the Jurkat cell line showed Ik-1, Ik-2, and Ik-3 as the major isoforms present, as reported previously (4). However, in whole cell lysates from patients who demonstrated overexpression of the dominant negative isoform Ik-6 (Fig. 1, patients 1, 35, and 37), significant amounts of Ik-6 protein were detected (Fig. 2). These data confirm the results of the RT-PCR analysis (Fig. 1).

To determine whether a genetic lesion could be correlated with overexpression of the dominant negative isoform Ik-6, Southern blot analysis was performed using human Ikaros cDNA as a probe. PsI digest of genomic DNA revealed differences in the restriction fragments between normal volunteers (n = 10) and patients with the dominant negative isoform Ik-6 [n = 4 (Fig. 3)]. A novel band indicated by an arrow was observed in DNA from the BV-173 cell lines.
line and in patients who showed overexpression of the dominant negative isoform Ik-6 (Fig. 1, patients 2, 15, 35, and 37). No gross abnormalities could be detected with BamHI and EcoRI digestion (data not shown).

**Discussion**

In the present analysis, RT-PCR was used to determine the expression levels of the Ikaros family genes, Ikaros and Aiolos, in bone marrow samples from adult ALL patients (n = 46; B-ALL = 41; T-ALL = 5). In 14 of 41 cases of adult B-ALL patients, Ikaros activity was reduced by overexpression of the dominant negative isoform Ik-6. Due to the limited amounts of bone marrow samples, immunoblotting analyses could only be performed on the samples from patients 1, 35, and 37 (Table 1). These experiments demonstrated that the dominant negative Ik-6 protein was stably expressed at high levels in those patients. PstI digests of genomic DNA revealed differences in the restriction fragments between normal volunteers (n = 10) and patients with the dominant negative isoform Ik-6 (n = 4). No gross abnormalities could be detected in those patients with BamHI and EcoRI digestion, suggesting that a mutation in the Ikaros locus may alter gene splicing, resulting in aberrantly high levels of Ik-6. It is interesting that we detect the same novel restriction fragments at a relatively high frequency, and we are currently determining which region of the locus is affected.

Because the related gene Aiolos was shown in mice to be a tumor suppressor gene in B cells (7), we expected that Aiolos activity might also be reduced in cases of B-ALL. Although Aiolos expression appeared to be decreased in several patient samples, it is not clear that this expression was aberrant. In the mouse, Aiolos is normally expressed at very low levels in pro-B cells and becomes greatly up-regulated at the pre-B-cell stage (6). Unfortunately, we did not have enough material to characterize the developmental stage of these leukemias. Therefore, we cannot make any conclusions about the significance of the low expression levels detected in certain patient samples. Nevertheless, it was clear that no dominant negative splice forms of Aiolos were expressed. Moreover, it is likely that even in the leukemic samples that showed normal levels of Aiolos expression, Aiolos activity was reduced through the dominant negative action of Ik-6, which can interact with Aiolos proteins and interfere with their DNA-binding capacity.

Ikaros and Aiolos are present together in large multiprotein complexes that have chromatin remodeling activity and contain proteins such as Mi-2/histone deacetylases or Brg-1/Swi-3/BAF60 (17, 18). The function of Ikaros and Aiolos in these complexes may be to recruit chromatin remodeling activity and histone deacetylases to the appropriate gene targets, thus enabling proper gene expression. The overexpression of dominant negative proteins such as Ik-6 may interfere with the ability of these complexes to be properly recruited to target genes. In the case of the M2 form of acute myeloid leukemia, the AML1-ETO fusion protein results in the inappropriate targeting of histone deacetylases (19, 20). Thus, aberrant gene expression resulting from inefficient or inappropriate targeting of histone deacetylases may be an important mechanism underlying leukemogenesis.

The results presented here contribute to a growing body of work showing that mutations that alter Ikaros activity correlate with hematological malignancies in humans (11–14). Interestingly, unlike the murine model (2, 3), in which these malignancies are confined to the T lineage, mutations in the Ikaros locus appear to contribute to malignancy in other hemopoietic lineages. This is likely to be due in some cases to the presence of other genetic lesions, such as BCR/ABL, which may cooperate with a mutation in Ikaros to target a cell that
would otherwise not be affected by a mutation in Ikaros alone. Characterizing these potential genetic interactions will be of future interest.

References

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Cancer Res 2000;60:4062-4065.

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