

Brief report

The *Ikaros* gene, a central regulator of lymphoid differentiation, fuses to the *BCL6* gene as a result of t(3;7)(q27;p12) translocation in a patient with diffuse large B-cell lymphoma

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The *BCL6* gene, isolated from the breakpoints of 3q27-associated chromosomal translocations, has been implicated in diffuse large B-cell lymphomas (DLBL). Here we describe the molecular characterization of novel t(3;7)(q27;p12) translocations in 2 patients with DLBL. Molecular genetic analysis of the breakpoint area involving *BCL6* revealed the presence of the *Ikaros* gene, a central regulator of

lymphoid differentiation that had been mapped to human chromosome 7 band p13-p11.1. As a molecular consequence of the translocation, the 5' regulatory region of the *BCL6* gene was replaced by the putative 5' regulatory region of the *Ikaros* gene, probably leading to deregulated expression of the *BCL6* gene throughout B-cell differentiation. Reverse transcription-polymerase chain reaction

(RT-PCR) and fluorescence in situ hybridization (FISH) analyses of a patient sample established that the t(3;7)(q27;p12) results in fusion of the *Ikaros* and *BCL6* genes. This study provides the first evidence that the *Ikaros* gene is rearranged in human hematopoietic malignant disorders. (Blood. 2000;95:2719-2721)

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Introduction

The *BCL6* gene has been implicated in the etiology of some non-Hodgkin's lymphomas (NHLs), especially diffuse large B-cell lymphomas (DLBLs).¹⁻⁵ The *BCL6* gene is located at 3q27 and has been isolated from recurrent breakpoint sites of chromosomal translocations in DLBLs. Rearrangement of the *BCL6* gene was found in as many as 40% of DLBLs and in 10% of a subset of follicular lymphoma (FCL).^{6,7} These alterations are thought to cause deregulated expression of the *BCL6* gene by means of a novel mechanism, promoter substitution—that is, the juxtaposition of heterologous promoters from different chromosome loci to the intact *BCL6* coding region.⁸

The chromosomal translocations involving *BCL6* are variable.⁹ In addition to the immunoglobulin (Ig) loci, 14q32 (IgH), 2p11 (IgLκ), and 22q11 (IgLλ), they also include 1q21, 2q21, 4p11, 5p13, 9p13, 11q23, 12p11, 12q13, and 15q21.^{7,9} Recently, the small G protein TTF on 4p11, a B-cell-specific transcriptional activator BOB1 on 11q23, and histone H4 on 6p21 have been isolated from cases or cell lines with 3q27 translocations.¹⁰⁻¹² Each gene of these translocations formed a fusion transcript with *BCL6* by removing the first *BCL6* noncoding exon, thus leaving this oncoprotein intact. We recently reported on 2 patients with DLBL with novel t(3;7)(q27;p12) translocations.¹³ We report here the case of a patient with DLBL whose *Ikaros* gene, a central regulator of lymphoid differentiation, fused to the *BCL6* gene as a result of t(3;7)(q27;p12) translocation.

Study design

5'-rapid amplification of complementary DNA (cDNA) ends (RACE)

First strand cDNA was generated by reverse transcription (RT) using the antisense primer 1 in *BCL6* exon 3: (5'-TGTCGACTCCGGAGACGAT-TAAGGT-3'), followed by the addition of dCTPs to the 3' end of the cDNAs and polymerase chain reaction (PCR) using 5' RACE abridge anchor primer (5'-GGCCACGCGTCTCGACTAGTACGGGGG GGGGG) and the nest antisense primer 2 in exon 2 (5'-ACTGGATACAGCTGTCCAG CC GCGA-3'). The diluted PCR products were further amplified with the abridged universal amplification primer (5'-GGCCACGCGTCTCGACTAG-TAC-3') and the nest antisense primer 3 from exon 2 (5'-CCAAAATTTT-GCTCAAAC-3'). The final PCR product was sequenced by using an automated 372 DNA sequencer (Applied Biosystems, Foster City, CA).

Genomic DNA and cDNA library construction

The genomic DNA library from patient 2's lymphoma samples and the cDNA library from a human B-cell lymphoma cell line, SP49, were constructed as described elsewhere.¹⁴ The genomic library (3 × 10⁵ plaque-forming units) was screened with a 4.0-kilobase (kb) *BCL6* major translocation cluster probe (Figure 2).³

Fluorescence in situ hybridization (FISH) analysis

FISH analysis with a YAC clone 19GA10¹⁵ upstream of the *BCL6* locus and a BAC clone (Genome Systems Inc, St Louis, MO), including the

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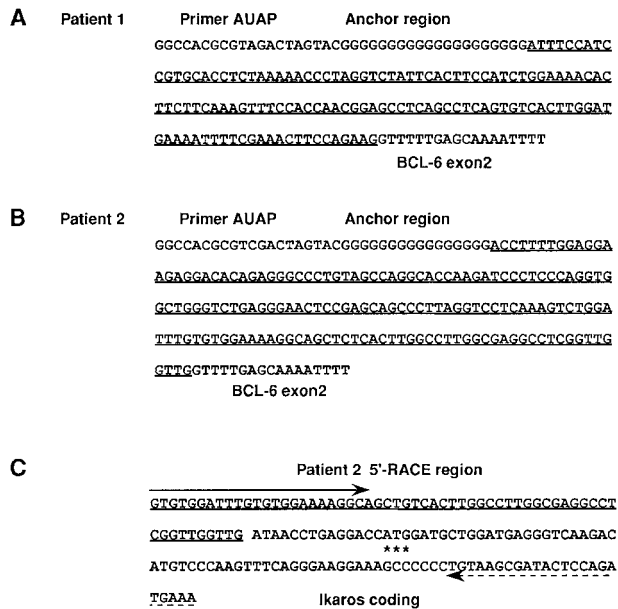


Figure 1. 5' RACE assay of patients 1 and 2 and RT-PCR analysis of 5' noncoding region of *Ikaros* gene. (A, B) The foreign sequences identified with the 5'-RACE method in 2 patients with DLBL with t(3;7)(q27;p12). (A) patient 1, (B) patient 2. The foreign sequences fused to *BCL6* sequence are underlined. GenBank accession no. AF180297. (C) Sequence of the same RT-PCR products of 4 B-cell lines obtained with a sense primer (shown by arrow) for the foreign sequence identified in patient 2 and an antisense primer (shown by dashed arrow) for the *Ikaros* coding region. The foreign sequence (underlined) in this patient was found to correspond to a part of the 5' noncoding region of the *Ikaros* gene. The translation initiation ATG codon of the *Ikaros* gene is indicated with 3 asterisks.

C-terminal portion of the *Ikaros* gene, was performed as described elsewhere.¹⁶

Results and discussion

To determine the molecular genetic basis for the 2 DLBL cases with t(3;7)(q27;p12) translocation,¹³ we first performed the 5' RACE assay of the total RNA samples from patients 1 and 2. As expected, sequence analysis of the resultant PCR products revealed that 5'-foreign sequences were fused to the *BCL6* exon 2 region, as shown in Figure 1A and B. However, a BLAST homology search revealed that these sequences did not bear any significant homology with any known sequences. We confirmed the presence of a specific-fusion transcript in each case by performing RT-PCR analysis with a sense primer for the foreign sequence identified by RACE and an antisense primer for the *BCL6* exon 3 region (data not shown).

To clone the genomic DNA fragments that contain the chromosomal breakpoint of t(3;7)(q27;p12), we next constructed patient 2's lymphoma sample-specific genomic DNA library in a λ DASH II phage vector. This screening allowed us to isolate 2 different genomic clones with insert sizes of 19 and 23 kb, as schematically illustrated in Figure 2. Restriction enzyme mapping and Southern blot analysis showed that the clone of 19 kb represented the *BCL6* germ-line allele and the clone of 23 kb represented the rearranged allele.

The 2 fragments (7-kb Bgl II-Kpn I and 2.5-kb Kpn I-Bgl II in Figure 2) derived from the 7p12 locus of the rearranged allele were used for Northern blot analysis to examine whether this region included transcribed exons. At least 2 hybridization bands of

approximately 5 to 8 kb were clearly detected by Northern blot analysis of a B-lymphoma cell line, SP-49 (data not shown). To isolate the *BCL6* partner transcript, the genomic Kpn I-Bgl II fragment of 2.5 kb was then used to screen an SP-49 random-primer cDNA library constructed in λ gt 10. BLAST homology search disclosed that the sequences of the 2 isolated cDNAs completely matched that of the human *Ikaros*, which was shown to play a central role in lymphoid differentiation.^{17,18}

To confirm whether the foreign sequence fused to the *BCL6* transcript was derived from the *Ikaros* gene, we performed RT-PCR analysis of the total RNA samples from 4 B-cell lines (NALM6, SP-49, Raji, and KMS-12-BM) without *BCL6* gene rearrangement at various differentiation stages by using a sense primer for the 5'-foreign sequence derived from patient 2 and an antisense primer for the *Ikaros*-coding exon 2 sequence. Sequence analysis of the amplified PCR products of the same size from these 4 cell lines led to identification of the fusion of the foreign sequence to the *Ikaros* region, thus establishing that the foreign sequence was indeed derived from the 5' noncoding region of the *Ikaros* gene (Figure 1C). Our observation is in agreement with an earlier report by Molnar et al¹⁹ that the human *Ikaros* gene is extremely large and complex, and it seems likely that as yet unidentified 5' noncoding exons are present in the human *Ikaros* gene. On the other hand, no apparent RT-PCR product could be amplified from any of the 4 above mentioned B-cell lines when a sense primer was used for the 5'-foreign sequence derived from patient 1 and an antisense primer for the *Ikaros*-coding exon 2 sequence. Thus, the origin of the foreign sequence (Figure 1A) from patient 1 remains unclear.

Finally, FISH analysis with a YAC clone 19GA10¹⁵ upstream of the *BCL6* locus and a BAC clone, including the C-terminal portion of the *Ikaros* gene, provided further evidence for our conclusion that the *Ikaros* and *BCL6* genes are fused as a result of the t(3;7) translocation in patient 2 (data not shown).

The *Ikaros* gene was previously assigned to chromosome band 7p13-p11.1,¹⁷ which is in agreement with our finding that this gene is rearranged in a human DLBL with t(3;7)(q27;p12). Because only 3 cases with t(3;7) have been reported in the literature,^{4,13} the frequency of involvement of the *Ikaros* gene in DLBL appears to be relatively low. Given the importance of the *Ikaros* gene in the ontogeny of the murine lymphopoietic system, it has been presumed that it may be involved in some forms of human severe combined immunodeficiency, leukemia, and lymphoma.^{18,19} In fact, it has recently been found that acute infant leukemia often express dysfunctional dominant negative *Ikaros* isoforms, implicating *Ikaros* in leukemogenesis.²⁰ This study provides the first evidence for the involvement of *Ikaros* gene rearrangement in human hematopoietic malignant disorders. Another group has recently

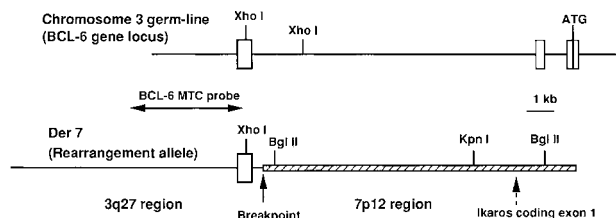


Figure 2. Schematic representation and restriction enzyme map of the chromosome 3 germ-line configuration (*BCL6* gene locus) and the chromosome 7 derivative (der 7). The 3q27 regions are shown by lines, and the 7p12 region by hatched bars. The breakpoint is indicated with a vertical arrow, and the presence of the *Ikaros* coding-exon 1 with a dashed vertical arrow. Exons I to III of the *BCL6* gene are boxed. A *BCL6* major translocation cluster (MTC) probe was used to screen a genomic DNA library constructed from patient 2.

performed the 5'-RACE analysis of 18 patients with DLBL carrying the *BCL6* gene rearrangement, and one of the *BCL6* partner genes was found to represent the *Ikaros* gene (Dr Moriyama, personal communication, June 1999). Together with the results of others, the chromosomal translocation between the *Ikaros* and *BCL6* genes has now been shown to recurrently occur in human DLBLs.

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