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ALL-1 partial duplication in acute leukemia

(leukemogenesis/direct tandem duplication/trisomy)

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ABSTRACT **The *ALL-1* gene, located on chromosome band 11q23, is fused to a variety of other genes by reciprocal chromosomal translocations present in 5–10% of human acute leukemias. We have recently reported the detection by Southern blot of *ALL-1* gene rearrangements in adult patients with acute myeloid leukemia lacking cytogenetic evidence of 11q23 translocations. These include 2 of 19 patients with normal karyotypes as well as 3 of 4 patients with trisomy 11. To characterize the abnormal *ALL-1* genes, we cloned the *ALL-1* rearrangements from two patients with trisomy 11. Characterization of the clones, together with Southern blot analysis, indicates that the *ALL-1* rearrangement in both patients is the result of a direct tandem duplication of a portion of the *ALL-1* gene spanning exons 2–6. The partial *ALL-1* duplication is also detected by Southern blot analysis in a patient with a normal karyotype. RNA PCR and DNA sequence analysis show that the partially duplicated *ALL-1* gene is transcribed into mRNA capable of encoding a partially duplicated protein. Partial duplication of *ALL-1*, in which a portion of a putative protooncogene is fused with itself, represents an additional genetic mechanism for leukemogenesis. Our findings suggest that the presence of trisomy in malignancy may sometimes indicate the partial duplication of a cellular protooncogene.**

Leukemogenesis often results from either the transcriptional activation of protooncogenes or the formation of fusion genes, which give rise to proteins capable of cellular transformation (1, 2). Both of these genetic mechanisms of hematologic malignancy are usually the consequence of chromosomal translocations, which juxtapose genes or genes and controlling elements normally found on different chromosomes. Although chromosomal translocations are found frequently in leukemia (3), many leukemias lack detectable chromosome rearrangements. In these cases, the genetic mechanisms of leukemogenesis remain unknown.

The *ALL-1* gene, located on chromosome band 11q23, is fused to a variety of other genes by reciprocal chromosomal translocations present in 5–10% of adult and pediatric acute leukemia cases (4–9). We (4, 5, 10) and others (6, 11, 12) have reported the cloning of *ALL-1* (also called *HRX, MLL*, or *Htrx*) and the molecular characterization of the t(4;11), t(6;11), t(9;11), and t(11;19) chromosomal translocations (5–8, 13–15), which constitute the most common of over 15 reciprocal translocations involving the *ALL-1* gene in acute lymphoblastic leukemia and acute myeloid leukemia (AML) (16). ALL-1, which shares regions of homology with the *Drosophila* trithorax protein, is a predicted 431-kDa protein with multiple zinc finger domains as well as AT hook motifs and a DNA methyltransferase motif thought to be involved in the recognition of methylated DNA (5, 6, 17). These structural features suggest that *ALL-1* may play a multifunctional role in transcriptional regulation.

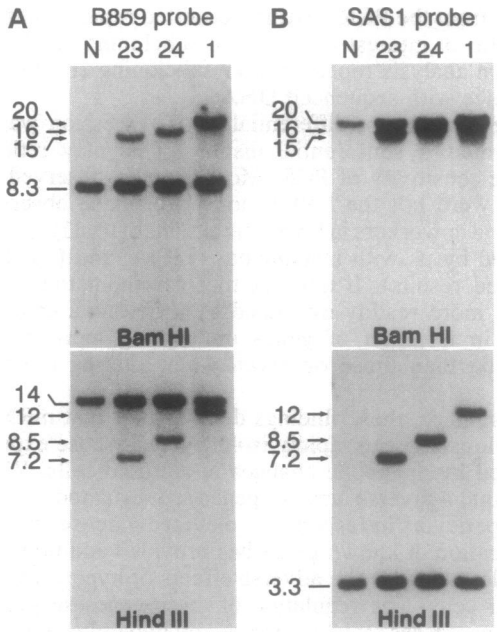

FIG. 1. Southern analysis of *ALL-1* gene rearrangements in adult AML patients without cytogenetic evidence of 11q23 translocations. The label above each lane corresponds to a unique patient identification number (18). Patients 23 and 24 had trisomy 11 as a sole cytogenetic abnormality, whereas patient 1 had a normal karyotype. Arrows indicate rearranged bands. N, normal control. (*A*) Blots examined with the B859 probe. Germ-line 8.3-kb (*Bam*HI) and 14-kb (*Hin*dIII) bands are indicated. (*B*) Blots examined with the SAS1 probe. Germ-line 20-kb (*Bam*HI) and 3.3-kb (*Hin*dIII) bands are indicated. The rearranged *Bam*HI band for patient 1 is presumably coincident with the germ-line 20-kb band. Rearranged bands detected with the SAS1 probe comigrate with the rearranged bands detected by the B859 probe.

We have recently reported the detection by Southern blot analysis of *ALL-1* gene rearrangements in adult patients with AML lacking cytogenetic evidence of 11q23 translocations (18). These include 2 of 19 patients with normal karyotypes as well as 3 of 4 patients with trisomy 11. To characterize the abnormal *ALL-1* genes, we cloned the *ALL-1* rearrangements in two of the patients with trisomy 11. Mapping and partial sequencing of the clones demonstrates a direct tandem duplication within a specific region of the *ALL-1* gene. The

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Abbreviation: AML, acute myeloid leukemia.

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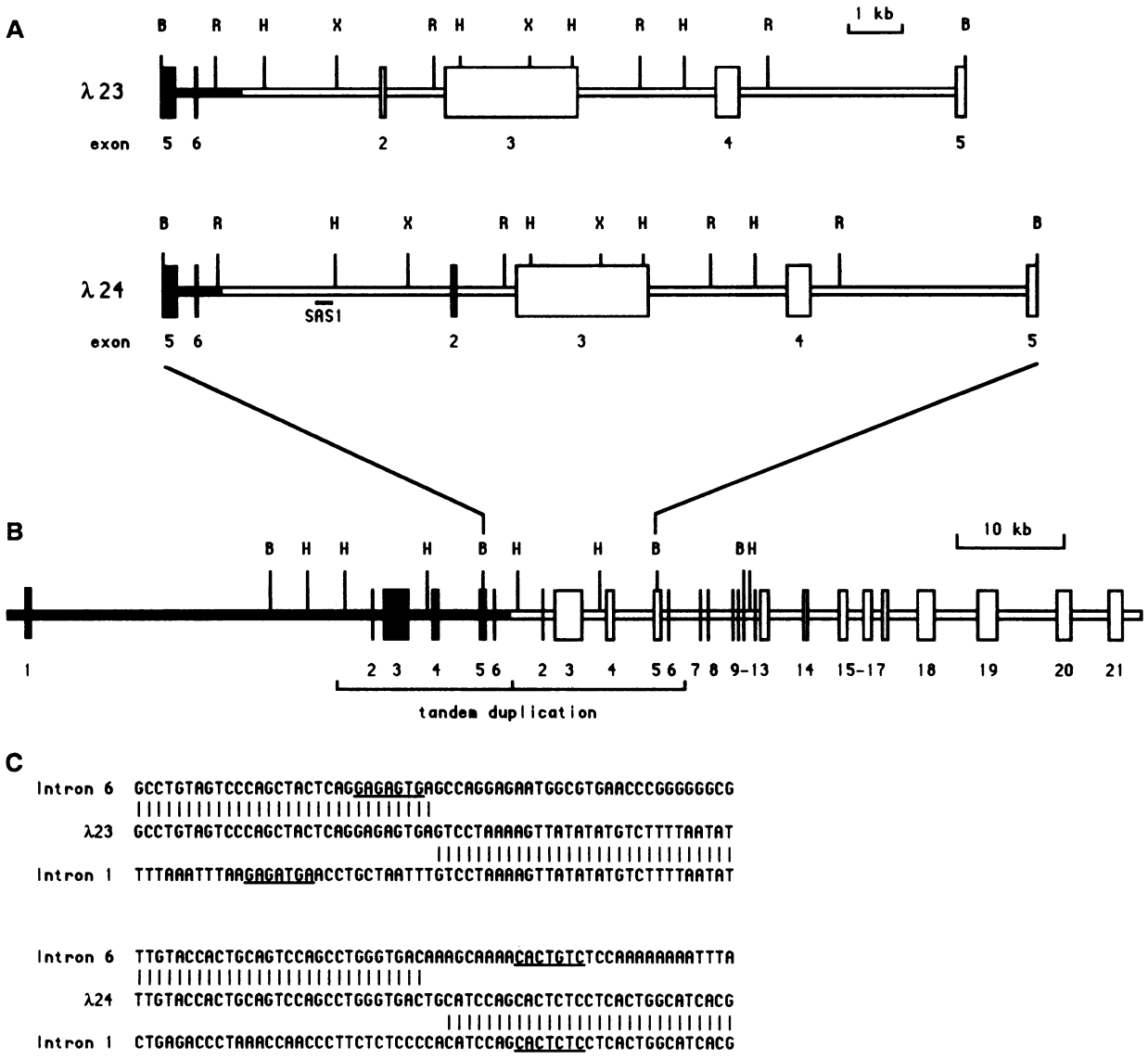

FIG. 2. Structure of partial duplication of the *ALL-1* gene. (*A*) Restriction enzyme maps of λ clones (λ23 and λ24) corresponding to rearranged *Bam*HI fragments from two AML patients with trisomy 11. Boxes represent *ALL-1* exon positions determined by subcloning and partial DNA sequence analysis. The junction point of the duplication is indicated by the juncture of the black and white bars. The position of the SAS1 probe is shown. B, *Bam*HI; R, *Eco*RI; H, *Hin*dIII; X, *Xba* I. (*B*) Proposed structure of the partially duplicated *ALL-1* gene contains a direct tandem duplication spanning exons 2–6. *Bam*HI and *Hin*dIII sites giving rise to bands detected on Southern blot (Fig. 1) are indicated. (*C*) DNA sequence across the junction points of clones λ23 and λ24 are aligned with sequences from introns 1 and 6 of the *ALL-1* gene. λ24 has a 2-bp extra nucleotide segment. Heptamer-like signal sequences near the junction points in both clones are underlined. Nonamer-like signal sequences are not present.

partial *ALL-1* duplication is also detected by Southern blot analysis in one of the patients with a normal karyotype. Partial duplication of *ALL-1*, in which a portion of a putative protooncogene is fused with itself, represents a distinctive genetic mechanism for leukemogenesis.

MATERIALS AND METHODS

Southern Blot Analysis. Genomic DNA was extracted from bone marrow aspirates of leukemia patients (19). Control DNA was isolated from peripheral blood. Approximately 8 μg of genomic DNA was digested to completion with *Bam*HI or *Hin*dIII. Restriction enzyme digests were separated by electrophoresis on 0.7% agarose gels and blotted onto positively charged nylon membranes. Southern blotting, probe radiolabeling, hybridization, and autoradiography were performed by standard techniques (20). Probes for patient and control samples are designated B859 (Fig. 1*A*) and SAS1 (Fig. 1*B*). B859 is an 859-bp *Bam*HI fragment of cDNA that spans the *ALL-1* breakpoint cluster region defined by exons 5–11 of the *ALL-1* gene (5, 18). SAS1 is a 289-bp DNA probe from intron 1 of the *ALL-1* gene derived from an *Xho* I/*Hin*dIII fragment by PCR. A single blot was prepared. After probing with SAS1, the blot was stripped and then probed with B859.

Molecular Cloning and Nucleotide Sequencing. Clones corresponding to the rearranged *ALL-1 Bam*HI fragments were isolated from bacteriophage λ EMBL3 libraries made from size-fractionated *Bam*HI digests of patient DNA. Recombinants were identified in phage libraries by filter hybridization using the B859 probe. Construction of libraries, screening, phage purification, and restriction enzyme mapping were done by standard techniques (20). Subclones from the λ phage recombinants were constructed in the pBluescript II plasmid vector. The DNA sequence of selected portions of subclones was determined by cycle sequencing using an Applied Biosystems 373A DNA sequencer. Programs from the Genetics Computer Group system were used for data analysis (21).

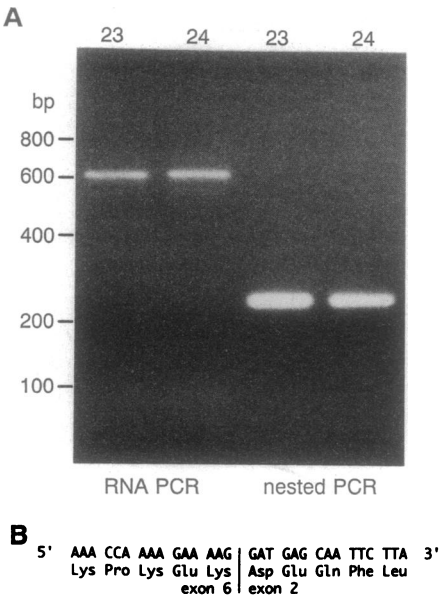

FIG. 3. RNA PCR analysis of trisomy 11 patient samples. (*A*) Ethidium-stained agarose gel of RNA PCR products (left-hand lanes) using oligonucleotide primers specific for the cloned *ALL-1* partial duplication. Right-hand lanes show the results of standard PCR amplification of an aliquot of the RNA PCR product using nested oligonucleotide primers. Discrete bands of the size predicted from the published *ALL-1* cDNA sequence (5) were detected for both RNA PCR (619 bp) and nested PCR (228 bp) products. Lanes are labeled with patient identification numbers (18). (*B*) Sequence analysis of nested PCR products shows an in-frame fusion of *ALL-1* exon 6 with exon 2. Amino acid translation is shown beneath the DNA sequence.

RNA PCR. Total cellular RNA was isolated using RNAzol (Biotecx Laboratories, Houston). Reverse transcriptase reaction and RNA PCR amplification were performed with thermostable r*Tth* DNA polymerase (Perkin–Elmer/Cetus). Nested PCR amplification was performed with *Taq* DNA polymerase. Oligonucleotide primers were used without further purification. Primers were 3.1c (5′-AGGAGAGAGTTTACCTGCTC-3′) from exon 3, 5.3 (5′-GGAAGTCAAGCAAGCAGGTC-3′) from exon 5, 6.1 (5′-GTCCAGAGCAGAGCAAACAG-3′) from exon 6, and 3.2c (5′-ACACAGATG-GATCTGAGAGG-3′) from exon 3. Primers used in reactions are as follows: (*i*) reverse transcriptase reaction, 3.1c; (*ii*) RNA PCR amplification, 5.3 and 3.1c; (*iii*) nested PCR amplification, 6.1 and 3.2c. Reverse transcriptase reaction was performed for 15 min at 57°C using ≈500 ng of RNA. RNA PCR amplification was performed for 35 cycles (95°C, 1 min; 53°C, 1 min; 72°C, 1 min). Nested PCR amplification was performed using ≈0.5 μl of the RNA PCR product for 30 cycles (95°C, 1 min; 60°C, 1 min; 72°C, 1 min). PCR products were analyzed by 2% agarose gel electrophoresis.

RESULTS AND DISCUSSION

Fig. 1*A* shows Southern blot rearrangements in the *ALL-1* gene for three adult patients with AML lacking cytogenetic evidence of 11q23 translocations. The rearrangements were detected with a cDNA probe (B859) that spans the *ALL-1* breakpoint cluster region (5, 18). Two of these patients (nos. 23 and 24) had trisomy 11 as a sole cytogenetic abnormality, whereas one patient (no. 1) had a normal karyotype (18). A single rearranged *ALL-1* band is seen for each patient in both *Bam*HI and *Hin*dIII restriction enzyme digests.

Clones corresponding to the rearranged *Bam*HI fragments from the two trisomy 11 patients were isolated and characterized. Each clone begins and ends with a portion of *ALL-1* exon 5 delineated by the *Bam*HI restriction site within this exon (Fig. 2*A*). The 5′ → 3′ order of *ALL-1* exons within each clone is 5-6-2-3-4-5. This unusual exon structure indicates that the *ALL-1* rearrangement in each patient is the result of a direct tandem duplication of a portion of the *ALL-1* gene spanning exons 2–6 (Fig. 2*B*). The junction point of this duplication fuses the 5′ portion of intron 6 to the 3′ portion of intron 1.

The precise junction points for the two clones are unique. DNA sequence across the junctions (Fig. 2*C*) shows a 2-bp extra nucleotide segment in clone λ24 and heptamer-like signal sequences (22) near the junction points in both clones λ23 and λ24. It has been postulated that the VDJ recombinase involved in immunoglobulin gene rearrangement may be directly involved in the t(4;11) reciprocal translocation involving the *ALL-1* gene (13). Although nonamer signal sequences are not found near the junction points of the *ALL-1* partial duplication, the presence of isolated heptamers raises the possibility that the VDJ recombinase may also play a role in the formation of the *ALL-1* direct tandem duplication.

We next examined the genomic DNA of the three AML patients with a probe from intron 1 (SAS1) designed to detect specifically the rearrangement associated with the *ALL-1* direct tandem duplication. The location of this probe is indicated in Fig. 2*A*. For all three patients, the SAS1 probe shows rearranged bands on Southern blot (Fig. 1*B*) that comigrate with the rearranged bands detected by the *ALL-1* breakpoint cluster region probe (Fig. 1*A*). This result indicates that the *ALL-1* partial duplication occurs in an AML patient (no. 1) with a normal karyotype, as well as in the two AML patients (nos. 23 and 24) with trisomy 11. Additional reported cases (18) of *ALL-1* rearrangements without 11q23 translocations lacked adequate material for further study.

Because the restriction fragments corresponding to the germ-line *ALL-1* breakpoint cluster region are also contained within the partially duplicated gene (see Fig. 2*B*), it is not possible to determine accurately from Fig. 1 the number of normal and abnormal *ALL-1* alleles in each patient. The analysis of alleles is further complicated by the mixture of normal and leukemic cells in the patient bone marrow samples that served as the source of DNA used in the Southern blots.

To determine whether the partially duplicated *ALL-1* gene is transcribed, RNA PCR was performed on samples from the two patients with trisomy 11 by using oligonucleotide primers specific for the cloned *ALL-1* duplication. Discrete bands of the predicted size were detected (Fig. 3*A*). Sequence analysis of nested PCR products (Fig. 3*B*) shows an in-frame fusion of exon 6 with exon 2. These results demonstrate that the partially duplicated *ALL-1* gene is transcribed into mRNA capable of encoding a partially duplicated protein.

The partial *ALL-1* duplication predicts a distinctive type of fusion protein in which a truncated polypeptide chain encoded by *ALL-1* exons 1–6 is fused near the amino terminus of the native ALL-1 protein. The duplicated region contains the AT hook motifs and the DNA methyltransferase motif of the protein. The structure of the partial duplication suggests that dissociation of ALL-1 amino-terminal domains from their normal protein environment is the critical structural alteration leading to *ALL-1*-associated leukemogenesis. This alteration of protein structure may lead to the alteration or inactivation of normal ALL-1 function, resulting in cellular transformation. Because the *ALL-1* gene is fused with itself, it follows that partner genes from other chromosomes are not necessary for involvement of *ALL-1* in leukemia.

Direct tandem duplications in the genome may arise by several different molecular mechanisms, including homolo-

gous recombination with unequal crossover, "slippage" during DNA replication, and repair of staggered breaks in DNA. Because the *ALL-1* gene participates in reciprocal translocations with many different chromosomal partners, one attractive mechanism for *ALL-1* partial duplication involves a t(11;11)(q23;q23) translocation occurring within the *ALL-1* gene locus. This model predicts a "reciprocal" *ALL-1* allele with a partial deletion corresponding to the duplicated region in the other allele. Southern blot experiments (data not shown) designed to detect a partially deleted *ALL-1* allele have thus far yielded negative results. Therefore, the molecular mechanism of the *ALL-1* direct tandem duplication remains to be elucidated.

We have reported previously a high incidence (three out of four cases) of *ALL-1* rearrangement associated with trisomy 11 as a sole chromosomal abnormality in AML (18). Trisomy 11 is a rare recurrent finding in AML, estimated to occur at a frequency of about 0.7% (Cancer and Leukemia Group B AML cytogenetic data base; C.D.B., unpublished data). Trisomy of other chromosomes is reported frequently in hematologic malignancy, sometimes in association with disease progression (3). Examples include trisomy 8 in AML and transformed chronic granulocytic leukemia, trisomy 21 in AML, and trisomy 12 in chronic lymphocytic leukemia (23). It has been postulated that trisomy, which occurs in somatic cells by nondisjunction, contributes to the neoplastic phenotype through a gene dosage effect (24). Our findings suggest that the presence of trisomy in malignancy may sometimes indicate the partial duplication of a cellular protooncogene.

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