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Structure and expression of the human trithorax-like gene 1 involved in acute leukemias

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ABSTRACT **The human trithorax-like gene 1 (Htrx1 gene) is disrupted in 11q23 translocations that are associated with acute leukemias. Sequencing of a partial human cDNA revealed an open reading frame encoding 1012 amino acids with extensive homology to the *Drosophila* trithorax protein, particularly in the zinc finger-like domains. Htrx1 gene appears to be unique in the human genome and has been conserved during evolution. Use of the human cDNA as a probe demonstrates that this gene is interrupted in both infant and adult acute myeloid (AML) and lymphoid (ALL) leukemia patients with 11q23 translocations. The structure of the Htrx1 gene around the breakpoints shows that this part of the human gene is interrupted by nine introns. As a result of the rearrangement, zinc finger domains are translocated in both ALL and AML patients. Expression studies reveal that the Htrx1 gene differentially expresses three transcripts within the normal lymphocyte cell lineage.**

The 11q23 region is involved in nonrandom chromosomal translocations with a number of different partner chromosomes (1). These rearrangements are observed in acute leukemias, especially—t(4;11), t(1;11), and t(11;19) translocations (2, 3) in acute lymphoid leukemias (ALL) and t(1;11), t(2;11), t(6;11), t(9;11), t(10;11), t(11;17), and t(X;11) in acute myeloid leukemias (AML) (4–8). The frequency of translocations involving this region is notably higher in infants (estimated to be 75%) (9–11) than in children and adults (≈5%) (12, 13). It has been shown that this region of the genome contains the human trithorax-like gene 1 (Htrx1 gene), which is interrupted in the translocation event in infant leukemic patients and in the cell line RS4;11 (14). To further characterize the Htrx1 gene, we isolated a portion of the corresponding cDNA, and we present the sequence of the putative Htrx1 protein in the region that is interrupted by the translocations. In *Drosophila* the trithorax gene (*trx*) is proposed to be a transcription factor and contains several zinc finger domains (15) that are highly conserved in Htrx1. This fragment of the human cDNA recognizes the translocations by Southern blotting in adult leukemia patients with t(4;11), t(6;11), t(9;11), and t(10;11) translocations as well as patient-derived cell lines carrying t(4;11) (RS4;11) (16) and t(X;11) (Karpas 45) (17). We have studied the expression of Htrx1 in normal human tissues and lymphocyte subsets and present evidence that this gene expresses at least three different transcripts within the hematopoetic cell lineage. We also describe the structure of this gene‡ around the breakpoints and present evidence that in a small series of adult ALL and AML patients, the breakpoints are clustered within less than 13 kilobases (kb) spanning four introns.

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MATERIALS AND METHODS

cDNA Library Screening. A *Kpn* I–*Eco*RI genomic fragment that had been shown to correspond to a transcribed region (14) was used as a probe to screen a human cortex cDNA library (Stratagene). One million plaque-forming units (pfu) were screened by standard techniques (18), and a single positive clone was identified. By using the Exassist/solr system (Stratagene), this clone was excised into plasmid pBluescript (pBS) (Stratagene).

Sequence Analysis. Nested templates were made of the cDNA in pBS by using exonuclease III/Mung-bean nuclease (Stratagene). Both strands of the cDNA were sequenced on an ABI 373A automated DNA sequencer (Applied Biosystems). Sequences were assembled by using Staden DNA sequence analysis on a Sun Sparcstation. Protein sequences were compared with the GenBank data bases by using the BLAST program (19).

Northern Blots and Southern Blots. These were prepared by standard techniques (14), and hybridizations were performed at 65°C in 0.5 M phosphate buffer (pH 7.2) and 7% SDS in a Hybaid rotating oven. Blots were exposed for up to 10 days at −70°C.

RESULTS

Identification of Htrx1 cDNA. A human brain cDNA library was screened, and a single clone of 3 kb was isolated (14). Sequencing revealed a single open reading frame of 1012 amino acids. To determine the number of related sequences to the Htrx1 gene in the human genome, the b fragment (see Fig. 3) of the cDNA was hybridized to *Eco*RI-, *Xba* I-, *Kpn* I-, and *Bam*HI-digested normal human peripheral blood DNA. The filter was washed at very low stringency [6× SSC (1× = 0.15 M NaCl/0.015 sodium citrate, pH 7) at 50°C], and no additional bands were detected, indicating that this gene is unique in the human genome (Fig. 1*A*). Moreover, the cDNA probe detected sequences on a Southern blot of DNA extracted from a variety of vertebrates, indicating DNA sequence conservation of this gene during vertebrate evolution (data not shown).

Protein Sequence of the Product of the Htrx1 Gene. The DNA sequence of the 3-kb fragment of Htrx1 cDNA was determined. Sequence comparison searches with the BLAST program of the translated cDNA unveiled marked similarity to the predicted *Drosophila* trithorax protein trx (15). Sequence alignment between the human and the *Drosophila* proteins show that the patterns of cysteine and histidine residues are strictly conserved. These cysteine-rich regions can be arranged into sequence motifs resembling zinc finger domains (Fig. 2*A*). Mazo *et al.* (15) have presented nine regions of the *Drosophila* protein and emphasized their

Abbreviations: ALL, acute lymphoid leukemia(s); AML, acute myeloid leukemia(s); Htrx1, human trithorax-like gene 1.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. L01986).

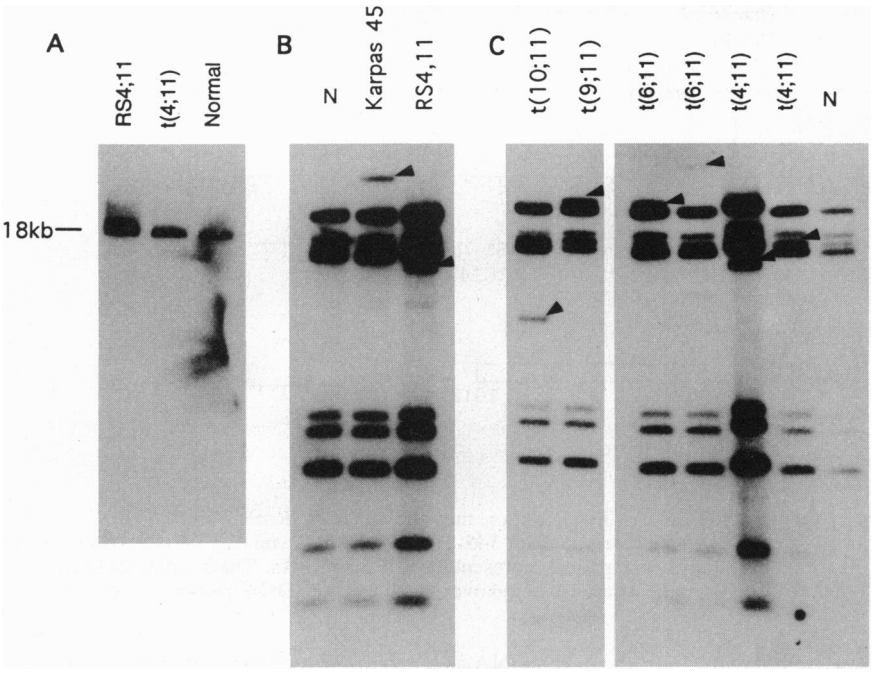

FIG. 1. Southern blot analysis of human DNA. Each lane contains 10 μg of peripheral blood DNA digested with the restriction enzyme, separated through a 1% agarose gel, and transferred onto Sure Blot membrane (Oncor). (*A*) Normal human, t(4;11) leukemic patient, and the RS4;11 cell line DNAs were digested with *Bam*HI. The blot was hybridized to the *Eco*RI fragment b of the Htrx1 cDNA. Washes were with 6× SSC at 50°C. (*B*) Analysis of *Eco*RI-digested DNA from a normal human (lane N) and the RS4;11 and Karpas 45 cell lines with t(4;11) and t(X;11), respectively. (*C*) Analysis of *Eco*RI-digested genomic DNA from a normal human (lane N) and adult leukemia patients with t(4;11), t(6;11), t(9;11), and t(10;11). *B* and *C* were probed with the 3-kb cDNA fragment, followed by washing with 1× SSC at 65°C. The rearrangements are indicated by arrowheads.

organization as putative zinc finger motifs. Fig. 2*A* shows that the human protein can also be arranged into the same zinc finger-like pattern. The arrangement of this portion of the protein also highlights the high number of conserved residues in addition to the cysteines and histidines (Fig. 2*B*). As pointed out by Mazo *et al.* (15), alternative putative zinc finger domains can be proposed because the cysteine residues allow the arrangement of several other zinc finger-like structures (Fig. 2*A*).

Genomic Structure of the Htrx1 Gene Around the Breakpoints. The intron–exon structure of the partial cDNA spanning the breakpoints was established by using the different *Eco*RI fragments of the cDNA (see Fig. 4) as probes on cosmid 116 (14) digested by *Eco*RI, *Bam*HI, and *Bam*HI/*Eco*RI. This approach determined the minimum number of introns contained within the 3-kb cDNA. To define exactly the position and length of each of these introns, direct sequencing of the cosmid was performed with oligonucleo-

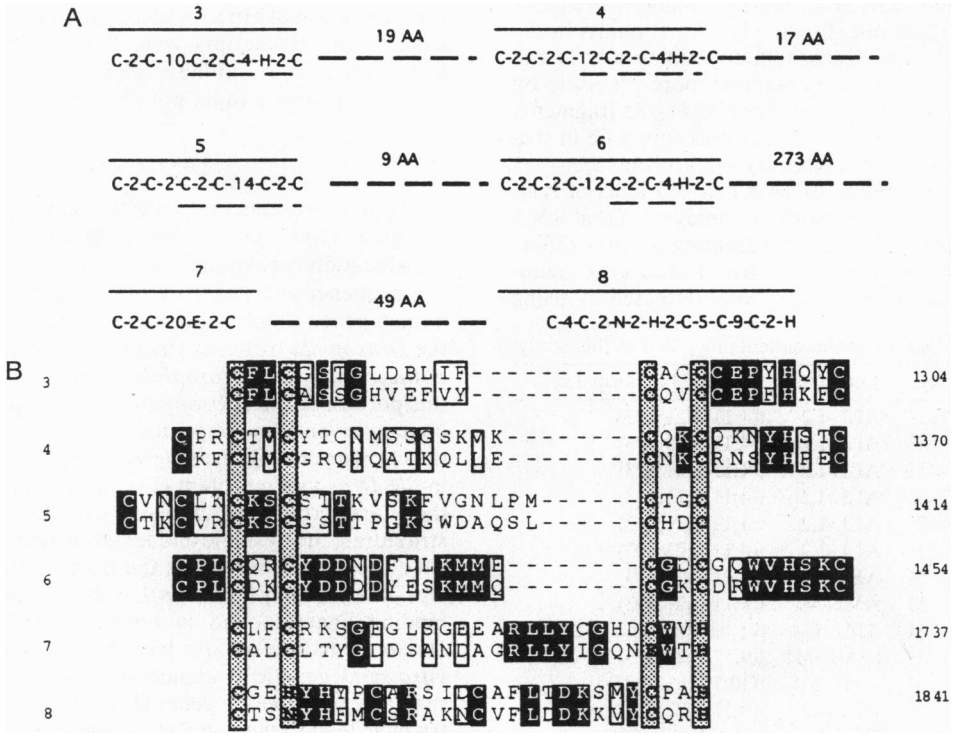

FIG. 2. (*A*) The pattern of cysteine (C) and histidine (H) residues in the cysteine-rich regions of Htrx1. The putative zinc finger structures are indicated by lines above the sequence; the numbers above each line refer to the *Drosophila* zinc fingers. The dotted lines indicate alternative zinc finger domains. (*B*) Putative zinc finger domains of the predicted Htrx1 protein. Alignment of the Htrx1 (*Lower*) to the proposed finger structures of the *Drosophila* trx zinc fingers (*Upper*). *Drosophila* domain numbers are given on the left; the last residue of each structure is given on the right. Amino acids that are identical between *Drosophila* and human are boxed in black; conservative changes are boxed.

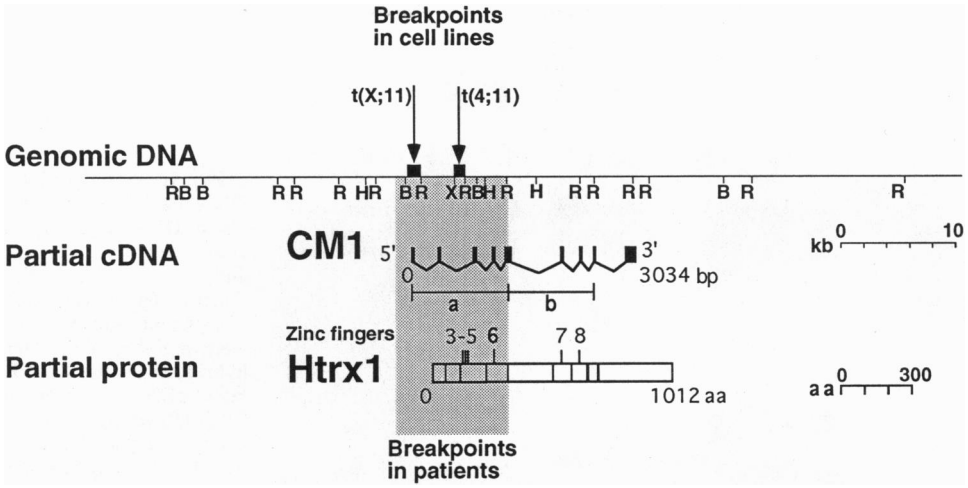

FIG. 3. Genomic map of the breakpoint region on chromosome 11q23. The restriction sites shown are R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; and X, *Xba* I. As described in *Results*, the exon/intron structure has been determined for 3-kb Htrx1 cDNA. The nine exons are boxed in black. The positions of the putative zinc fingers (nos. 3–8) are indicated on a schematical representation of the protein. The position of breakpoints in the cell lines has been mapped accurately, while the breaks in the patients are clustered over 13-kb genomic DNA shown by the shaded area on the diagram.

tides defined on the cDNA sequence. These results are summarized in Fig. 3.

Patient Studies. Clustering of the breakpoints on chromosome 11q23 has been demonstrated in infant leukemic patients with t(4;11) and t(9;11) translocations by using genomic probes derived from cosmids spanning the region (15, 20–22). The 3-kb cDNA used as a probe on Southern blots of adult leukemia cell DNA detects rearrangements in patients with t(4;11) (six of seven patients studied), t(6;11) (two of two), t(9;11) (one of one), and t(10;11) (one of one) with the enzyme *Eco*RI (Table 1; Fig. 1 *B* and *C*, and illustrated in Fig. 3). The single patient (no. 7) with t(4;11) in whom the rearrangement was not demonstrated showed the rearrangement with a more centromeric probe (data not shown). The breakpoints in the two cell lines RS4;11 and Karpas 45 containing t(4;11) and t(X;11), respectively, have been mapped more precisely by using a number of restriction enzymes and *Eco*RI fragments of the cDNA. In these cell lines, the breakpoints lie in two different introns and are separated by ≈5 kb (Fig. 3).

Expression. The expression of Htrx1 in cell lines of lymphoid origin was assessed by Northern analysis. Total RNA from T and B lymphocyte lines representing various differentiated states were separated and transferred onto membrane, and Htrx-specific sequences were detected by using the 3-kb cDNA as a probe. Three transcripts of 15, 12.5, and 11.5 kb were detected in the mature T-cell lines Jurkat and HUT78 and in the immature T-cell line CEM. Likewise, all three transcripts were expressed in the IgM-secreting B-cell line Namalwa. In contrast only the largest transcript was present at detectable levels in the murine pre-B-like cell line 70/3. 70/3 cells can be induced to differentiate towards a more mature B-cell phenotype by treatment with bacterial lipopolysaccharide (LPS) (24). As shown in Fig. 4, 6 hr of treatment with a final concentration of 10 μg of LPS per ml up-regulates the level of the 15-kb Htrx1 transcript and stimulates the expression of the 12.5-kb mRNA. In addition, the expression of Htrx1 was analyzed in normal human adult tissues, and transcripts were detected in brain, pancreas, liver, lung, heart, kidney, and skeletal muscle but were absent in placenta (data not shown).

Table 1. Cytogenetic status of the patients included in this study

Patient	Age, yr	Sex	Leukemia	11q23 abnormality
1*	79	F	ALL-L2	t(4;11)(q21;q23)
2	12	F	ALL-L2	t(4;11)(q21;q23)
3	43	M	ALL-L2	t(4;11)(q21;q23)
4	53	F	ALL-L2	t(4;11)(q21;q23)
5	39	F	ALL-L2	t(4;11)(q21;q23)
6*	44	F	ALL-L2	t(4;11)(q21;q23)
7	28	M	ALL-L2	t(4;11)(q21;q23)
8*	52	M	AML-M4	t(6;11)(q27;q23)
9	45	M	AML-M4	dir ins (6:11)(q27;q13q23)
10*	59	F	tAML-M1	t(9;11)(p22;q23)
11*	27	M	AML-M5	t(10;11)(p12;q23)
RS4:11†	<1	F	ALL-L2	t(4;11)(q21;q23)
Karpas 45‡	7	M	T-ALL	t(X;11)(q13;q23)

L2, M1, M4, M5 are French–American–British subtypes of leukemia. T-ALL, T-cell ALL.
*Previously described by Kearney *et al.* (23).
†Previously described by Stong *et al.* (16)
‡Previously described by Karpas *et al.* (17).

DISCUSSION

This report presents the predicted partial protein sequence of the gene Htrx1 in the region of the leukemia-associated translocation breakpoints on chromosome 11q23. Comparative sequence analysis shows that we have cloned part of the human gene, which contains extensive protein homology to the *Drosophila* trithorax (trx) protein. The overall homology in this region with *Drosophila* trx protein is 50%. Moreover, this portion of the *Drosophila* homologue has been shown to contain several zinc finger motifs that are highly conserved in the human gene. Of the nine zinc finger-like motifs described in the *Drosophila* protein (15), six are present in the part of the human protein that was analyzed. In these zinc finger-like structures, the cysteine and histidine residues are conserved. Like the *Drosophila* gene, the human zinc finger motifs are Cys-Xaa-Xaa-(Cys or His). This is similar to the DNA binding fingers found in hormone receptors (15, 25). In *Drosophila* these motifs have been shown to bind zinc *in vitro*, and genetic evidence suggests that trx is a positive regulator of homeotic genes (15, 26). These characteristics of trx have lead to the conclusion that this gene product is most likely a transcription factor. The disruption of the *trx* homologue in humans could lead to the deregulation of homeotic genes involved in hematopoetic ontogeny (27, 28), resulting in a malignant phenotype in both myeloid and lymphoid lineages. This mechanism has been described for the t(10;14)

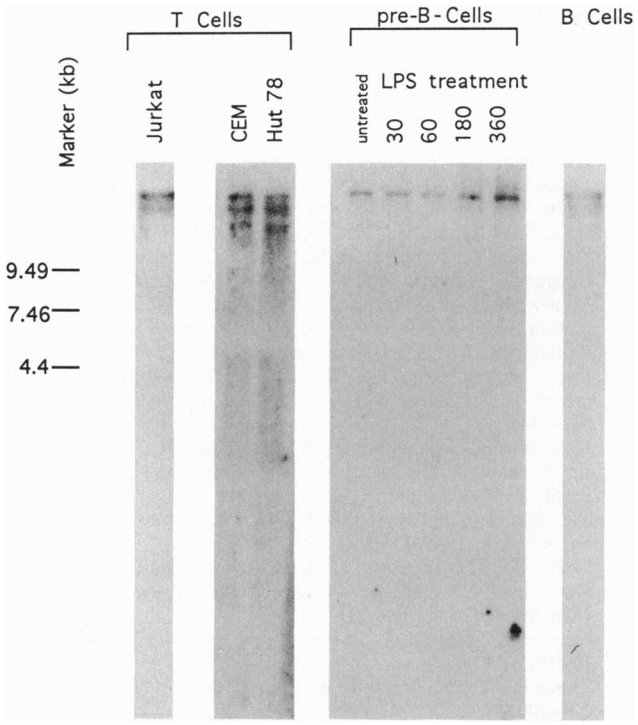

FIG. 4. Northern blot analysis of Htrx1 in mature T cells, mature B cells, and in pre-B-cells treated with LPS. Total RNA (15 μg) purified from the indicated cell lines was separated on formaldehyde/1% agarose gels, transferred to Hybond-N membrane (Amersham), and probed with the 3-kb fragment of Htrx1. Size estimates were determined relative to a series of RNA marker standards (BRL) that were run in parallel.

translocation associated with T-cell ALL, which results in the deregulation of the homeotic gene *HOX11* (29, 30).

We have demonstrated Htrx1 gene rearrangements in leukemic cells from both adult and infant patients (14), in both acute lymphoid and acute myeloid leukemias, and in both primary and therapy-related leukemias. The disruption of this gene in so many different clinical settings suggests an important role for Htrx1 in hematopoeisis and indicates that altered expression of this gene may be fundamental in leukemogenesis. The leukemia-associated 11q23 translocations involve a number of different partner chromosomes, and in general each translocation is associated with a particular leukemic phenotype. The oncogenic role of the partner chromosomes remains uncertain. However, the expression of fusion transcripts and production of chimeric proteins have been described in other leukemia-related translocations (31, 32). If fusion products are produced as a consequence of these translocations, the reciprocal genes from the partner chromosomes may determine the cell lineage expressing altered Htrx1 expression and the consequent leukemic phenotype.

The position of the breakpoints in both the cell lines and patients results in the loss of at least two of the six identified zinc fingers from the Htrx1 protein. Alternative splicing of a *Drosophila* transcription factor CF2 during development, leading to variations in the zinc fingers, results in the recognition of different DNA binding sites (33). Point mutations within the zinc finger domain of the human Wilm tumor gene *WT1* at a residue thought to be critical for DNA binding capacity and site specificity have been identified in Wilm tumors (34). The loss of zinc finger motifs from Htrx1 will alter the DNA binding and/or the specificity of the product whether it is a truncated or chimeric protein. The cloning of Htrx1 transcripts from patients with the various 11q23 translocations will elucidate whether Htrx1 fusion transcripts are expressed and may lead to the identification of chimeric proteins.

RNA (Northern) blot analysis using the cDNA spanning the breakpoint as a probe reveals three polyadenylylated transcripts (15, 12.5, and 11.5 kb); all three are expressed in mature and immature T-cell lines (Jurkat, HUT78, and CEM) and in a mature B-cell line (Namalwa). Only the largest of the transcripts was detectable in an immature B cell line (70Z/3), with the 12.5-kb mRNA appearing after 6 hr of LPS treatment. 70Z/3 cells exhibit features typical of the early stages of B-lymphocyte differentiation. They constituitively produce μ heavy chains without detectable light chain synthesis. Upon exposure to LPS, however, the cells initiate expression of κ light chain genes; subsequently, surface immunoglobulin molecules appear on the cell surface within 12 hr (35). Whether there is a relationship between the expression of the Htrx1 protein and the progression of pre-B-cells to a more mature phenotype remains unknown. Other studies (21, 22) have identified similar size transcripts in leukemic cell lines with the t(4;11) translocation. Cimino *et al.* (22) described an altered 11-kb transcript in cells carrying the t(4;11) translocation. This transcript is of a similar size to a normal splice variant of Htrx1, but because of the difficulty of measuring accurately the size of such a large mRNA, we do not know if these transcripts are identical. The presence of multiple introns identified in this study suggest that the Htrx1 gene may be alternatively spliced. Different splice variants may be preferentially expressed in different lymphocyte populations and may encode proteins that exhibit altered activities or DNA binding specificities as is described for CF2 (33). The possible role of alternatively spliced Htrx1 products in lymphocyte ontogeny awaits further studies on the structure of the gene to identify all of the different splice variants and their pattern of expression during ontogeny.

Note. Tkachuk *et al.* (36) and Gu *et al.* (37) have recently identified the same gene, which they have named human trithorax (HTX) and ALL-1, respectively. They have shown that it is rearranged in infant leukemia patients with t(4;11) and t(11;19) and have demonstrated fusion transcripts with chromosome 4 (AF4) and chromosome 19 (ENL) genes in cell lines carrying the translocations.

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