A Specific Deletion in the Breakpoint Cluster Region of the ALL-1 Gene Is Associated with Acute Lymphoblastic T-Cell Leukemias

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ABSTRACT

A variety of chromosomal translocations to the ALL-1 gene are regularly observed in acute leukemias and are thought to play a key role in the leukemogenic process. Chimeric proteins encoded by the breakpoint regions of the derivative chromosomes have been proposed to be the relevant oncogenic agents. In addition, internal duplications of the ALL-1 gene have been observed in patients with specific acute myeloid leukemias. Thus, it has been hypothesized that oncogenic variants of the ALL-1 protein may be generated by both chimerization and self-fusion, but the critical structural features ending the altered proteins with their oncogenic potential are still unknown. Here we describe structural alterations of the ALL-1 gene observed in three patients presenting with acute T-cell leukemia (ALL) without chromosomal translocations or self-fusions of the ALL-1 gene. These unrelated patients carried an internal deletion in one of the two alleles of the ALL-1 gene that eliminated parts of introns 7 and 8, together with exon 8. The deletion was found in 3 of 74 ALL patients, but not in acute myeloid leukemias, follicular lymphomas, or peripheral blood leukocytes from healthy donors. One ALL patient showed the deletion at diagnosis but no longer at remission or at 9 months after remission. These findings support the hypothesis that the ALL-1 protein may be converted to an oncogenic variant, not only by chimerization or self-fusion, but also by deletion of sequences coded by exon 8. They further suggest that these three different types of structural alterations of the ALL-1 protein may each cause a distinct disease phenotype. Alternatively spliced mRNA species omitting exon 8 were observed in 14 of 24 ALL patients without detectable macroscopic alterations of the ALL-1 gene and also in peripheral blood leukocytes from healthy donors.

INTRODUCTION

The ALL1 gene 1 (ALL-1), also called the MLL (mixed lineage leukemia) or human trithorax (Htxrl or HRX) gene, participates in a broad array of chromosomal translocations associated with ALLs and AMLs (1-5). A subset of these leukemias, the high-risk infant leukemias, are characterized by their occurrence in very early childhood and a very poor prognosis. The majority of these cases show either the t(4;11) or the t(11;19) translocation to the ALL-1 gene and a disease phenotype characterized by mixed lineage properties of the leukemic blasts displaying both pro-B lymphoid and myeloid markers, very high WBC counts, responsiveness to chemotherapy at diagnosis, and resistance after first relapse (6-14, 16-20). The t(9;11) translocation to the ALL-1 locus has been regularly found in childhood AML (21), and other translocations to the ALL-1 gene have been observed in AML patients (reviewed in Ref. 22), particularly in adults with secondary AML occurring after chemotherapy of primary tumors with topoisomerase inhibitors (23-26).

Cytogenetic studies thus far revealed 29 different chromosomal translocations to the q23 region of human chromosome 11, and a fraction of these have been identified as translocations to the ALL-1 gene (22). The ALL-1 cDNA (3, 4) and cDNAs for a number of translocation partner genes from different chromosomes have been cloned and characterized, including the genes AF-Ip (27), AF-Iq (28), AF-4 (3), AF-6 (29), AF-9 (17), AF-IO (30), AF-17 (31), ELL (32), ENL (4), and the AFX1 gene located on the X chromosome (33, 34). Due to this unusually broad range of translocation partners and the generation of chimeric breakpoint proteins in the majority of cases, the hypothesis was proposed that chimeric breakpoint proteins are the oncogenic agents responsible for the malignant transformation. The hypothesis further specified that the NH2-terminal portion of the ALL-1 protein, contained as the smallest common denominator in all chimeric proteins encoded by the various derivative 11 (der11) chromosomes, was an essential part of the oncogenic principle (35-37). It was anticipated that these chimeric proteins may act as dominant-negative inhibitors of the intact ALL-1 protein contributed by the unaltered chromosome 11, which was consistently expressed in the leukemic blasts, possibly by forming heterodimers interfering with the normal ALL-1 function.

The wild-type ALL-1 protein sequence deduced from cDNA specifies a polypeptide of close to 4000 amino acids with sequence homology to the drosophila trithorax protein (dTRX), an important regulator of early development in Drosophila (38). It contains an AT-hook DNA-binding motif and a potential cysteine-methyltransferase domain in its NH2-terminal portion and a bipartite zinc-finger motif in its COOH-terminal portion (2, 4, 37, 39). The AT-hook DNA-binding motif and the methyltransferase domain are present in all chimeric breakpoint proteins produced from the der11 chromosomes of the various translocations. However, it is not obvious how this part of the protein may generate the oncogenic potential or act as a dominant-negative inhibitor of the wild-type ALL-1 protein. One proposed explanation was that the simple separation of the NH2- and COOH-terminal portions of the ALL-1 protein might suffice to generate the oncogenic function and that the COOH-terminal portions of the chimeric proteins derived from the various translocation partner genes may contribute little or nothing to their transforming potential (37).

Recently, internal duplications of exons 2 to 6 or 2 to 8 of the ALL-1 gene have been reported in AML patients with trisomy 11 (40, 41). This observation suggests that oncogenic variants of the ALL-1 protein may also be generated by self-fusions causing duplication of parts of the ALL-1 protein. However, an actual transforming function of the self-fused proteins has not yet been demonstrated directly, and the portions of the protein generating the oncogenic potential have not been defined with precision. Here we describe a third structural alteration of the ALL-1 protein, a deletion of sequences coded by exon 8, that may generate transforming potential. The omission of these protein sequences can be caused either by a genomic deletion encompassing...
passing exon 8 or by unidentified mechanisms leading to the omission of exon 8 in an alternatively spliced mRNA without a concomitant genomic deletion. Alternatively spliced breakpoint mRNAs generated by omission of one or several exons from each translation partner gene have been reported previously by other authors for various translocations to the ALL-I gene (3, 4, 6, 17, 35, 39, 42). In addition, a number of cDNAs corresponding to alternatively spliced variants of the intact ALL-I mRNA have been reported (2-4, 43, 44), including a cDNA lacking the coding sequences for exon 8 (43). However, to date a simple deletion of coding sequences of the ALL-I protein had not been proposed as a potential oncogenic mechanism. If confirmed, the present report of a correlation between the occurrence of a genomic deletion of exon 8 and the incidence of T-ALL may help to define the potentially oncogenic features of variant ALL-I proteins with greater precision.

MATERIALS AND METHODS

PCR Primers and Oligonucleotide Probes. Primers 5'-3' (5'-CCTAAAACACTCTAGTAGGAG-3'), 7' (5'-CAGGATGGAGTCCCA-CAG-3'), and 10' (5'-CTGGTGATAGCTGTTTCGG-3') bind to exons 5, 7, and 10, respectively; Primers 8-3' (5'-GGACTATTTAAGGCTCACC-3') and 9-5' (5'-ATCTTTGAGAGCTATTTGCC-3') bind to introns 7 and 8 of the ALL-I gene. Oligonucleotide delta8-3' (5'-GGTCTAGGTGTC- GAGGCT-3') contained sequences from both introns 7 and 8 and formed stable hybrids in Southern blot experiments selectively with amplimers from the deletion allele but not from the intact allele.

Patient Materials and Cell Lines. Leukemic cells used in this study were collected from children and adolescents enrolled in treatment protocols of the German BFM study group, which had been approved by institutional review boards. All patients and their parents or guardians gave informed consent for treatment and for collection of material for biological studies. Our panel comprised 74 pediatric patients with variant types of ALL including pro-B-, pre-B-, common, and T-ALL. Material from 50 patients was available as cryopreserved leukemic bone marrow samples, and leukemic cells from 24 patients were isolated freshly from bone marrow biopsies at diagnosis. Patients A1, A8, and A15 were diagnosed as T-ALL, and relevant patient data are listed in Table 1. Genomic DNA obtained from fresh bone marrow samples of 10 adult AML patients was provided by Drs. M. Gramatzki and G. Eger (Medical Department III, University of Erlangen-Nürnberg). Genomic DNA obtained from lymph node biopsy material from 10 FLs was kindly donated by Dr. A. Polack (Gesellschaft für Strahlen- und Umweltforschung, Munich, Germany). PBLs were obtained from healthy volunteers after informed consent according to approved standard protocols for vena puncture. The Namalwa cell line, a well described B-lymphoblastoid cell line, was obtained from the American Type Culture Collection (Rockville, MD). Isolation of Genomic DNA and Southern Blot Hybridizations. MNCs (5 x 10^5) from peripheral blood or bone marrow aspirates from ALL patients were collected by Picoll gradient centrifugation and resuspended in ice-cold PBS. The cells were lysed, and genomic DNA was isolated as described (15). The genomic DNA was then digested with restriction enzymes or used directly for PCRs. Southern blot hybridizations were performed using standard procedures (45).

PCR Assays with Genomic DNA. Genomic DNA (50 to 100 ng) from PBLs of healthy donors or biopsy samples from ALL, AML, and FL patients was amplified for 35 cycles in a PCR reaction with denaturation for 30 s at 94°C, annealing for 30 s at 58°C, and extension for 90 s at 72°C in each cycle. PCR products were electrophoresed in a 1.3% Seakem agarose gel next to a DNA size marker (pBR322 × Hinf I). Amplified DNA fragments were isolated from the gel and cloned into pBluescript plasmids (Stratagene) for further analysis. Comparison of several independently generated cDNA sequences from the same region indicated that the error rate in our experiments was smaller than 1:10^4 nucleotides. Rearrangements of the ALL-I gene in genomic DNA from leukemic cells were studied in a PCR assay using the primers 73, 8-51, and 9-54. This assay allowed the simultaneous detection of intact ALL-I alleles and alleles carrying a deletion of exon 8 in one reaction tube. Fragments longer than 1000 bp were amplified poorly in this PCR assay. Human placenta DNA was used as a negative control.

Assessment of the Sensitivity of the Deletion-specific PCR Assay. To evaluate the sensitivity of the deletion-specific PCR assay, 500 ng of placenta DNA were mixed with genomic DNA from patient A1 in various dilutions (500 ng to 0.5 pg, with 5 pg taken as the DNA content of a single cell). The deletion-specific PCR assay with primers 73-8-51, 8-51, and 9-54 was performed, and resulting amplimers were separated by gel electrophoresis and blotted to a nylon membrane. After transfer, nucleic acids were immobilized by UV cross-linking and hybridized with the radiolabeled oligonucleotide delta8-3', which recognized exclusively the amplimers from the deleted allele.

RT-PCR Reactions with mRNA from Biopsy Material. MNCs (2 x 10^5) from ALL patients and PBL from healthy donors were collected, and mRNA was isolated using the QuickPrep Micro mRNA purification kit (Pharmacia). Hexamer-primed cDNA was synthesized in 20-µl reactions containing 0.1 µg mRNA, 200 pmol random hexamer primer, 4 µl of 5 × first-strand synthesis buffer (Life Technologies, Inc.), 2 µl of 100 mm DTT, 4 µl of 2.5 mm stock solutions of each of the four deoxynucleotide triphosphates, 200 units Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) and 2 units of the RNase inhibitor RNasin (Serva). Reactions were incubated for 60 min at 42°C and diluted to 120 µl. Five µl were used as template and were amplified through 40 cycles using a "touch down" PCR program (initial cycle: denaturation for 30 s at 94°C, annealing for 30 s at 65°C, and extension for 60 s at 72°C; the annealing temperature was then lowered by 0.5°C in every subsequent cycle until 54°C was reached in cycle 26; the last 14 cycles were performed with an annealing temperature of 54°C). PCR products were electrophoresed in a 1.3% Seakem agarose gel next to a DNA size marker (pBR322 × Hinf I). Amplified DNA fragments were isolated and cloned into pBluescript plasmids (Stratagene).

RESULTS

Exon 8 of the ALL-I Gene Is Deleted in Three Independent ALL Patients. Southern blot experiments with DNA from MNCs of childhood ALL patients were performed routinely to search for translocations to the ALL-I gene. Patient A1 showed an intense 3-kb EcoR1 restriction fragment in addition to the 5.2-, 4.7-, and 4.2-kb fragments

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resulting from the intact ALL-1 gene (Fig. 1). Further analysis of this fragment revealed a genomic deletion of 1634 bp eliminating exon 8 plus flanking sequences on either side. A specific PCR assay for exon 8 was designed (Fig. 2A), and DNA was prepared from cryopreserved biopsy material of 74 ALL, 10 AML, and 10 FL patients and from MNCs of 20 healthy donors. An indistinguishable deletion was detected in the DNA of ALL patients A1, A8, and A15 (Fig. 2B), but none of the AML and FL patients and healthy donors in our panel showed this deletion. Subsequent review of the case histories showed that all three patients had presented with T-ALL. Sequence analysis of the PCR amplimers from patients A1, A8, and A15 showed an identical deletion in all three of these unrelated patients (Fig. 2A). In all three cases, the deletion occurred in only one of the two alleles at palindromic sequences located in introns 7 (5'-TGATCA-3') and 8 (5'-CTCGAG-3'), respectively (Fig. 2C). As a result of the deletion, sequences from introns 7 and 8 were fused (5'-TGATCTCGAG-3'), and large parts of introns 7 and 8 were deleted together with exon 8. An XhoI restriction site (5'-CTCGAG-3') located adjacent to the point of fusion in intron 8 remained intact and was used for diagnostic purposes. The regions from introns 7 and 8 engaged in the recombination showed no extensive sequence similarities, suggesting that the deletion was generated by illegitimate recombination through an unknown mechanism.

The Deletion Is Associated with the Leukemic Blasts. Biopsy material from patient A1, taken both at diagnosis and during therapy, was available. After 33 days of induction therapy, the patient achieved a first remission, and the deleted allele was no longer detectable in the PCR assay (Fig. 3A). To evaluate the sensitivity of the assay, serial 10-fold dilutions of genomic DNA from patient A1 were mixed with constant amounts of placenta DNA and analyzed (Fig. 3B). Although the PCR reactions were performed under semicompetitive conditions, the deleted allele was still detectable after a 10,000-fold dilution of the patient DNA (Fig. 3B). Thus, the deleted allele disappeared for patient A1 together with the leukemic blasts at remission. Patient A1 was still in remission after 9 months of initial therapy, and another biopsy sample was obtained at this time. The deletion-specific PCR product was not detected, whereas the product from the intact allele was obtained as an internal positive control (Fig. 3A). The hematopoietic system of the patient had largely recovered at this time; therefore, it is considered unlikely that the concordant disappearance of the leukemic blasts and the deletion-specific PCR product was a simple coincidence and was due to the simultaneous disappearance of a population of cells different from the leukemic blasts that carried the deletion. Such a population should have recovered from therapy together with the majority of hematopoietic cells, and the deletion-specific PCR product should have reappeared.

An Alternative RNA Species Lacking Exon 8 Sequences Is Present in Healthy Donors and ALL Patients in the Absence of Detectable Genomic Alterations. mRNA was isolated from MNCs of 24 ALL patients lacking alterations of the ALL-1 locus and from 20 healthy donors. cDNA was synthesized from each mRNA sample and used in RT-PCR experiments with a pair of primers complementary to exon sequences flanking the breakpoint cluster region of the ALL-1 gene (Fig. 3C). A PCR product corresponding to a variant, shorter mRNA species was observed in 14 of 24 ALL samples. This product represented a variant mRNA species lacking exon 8 (Δ8; Fig. 4A), as shown by cloning and sequence analysis. All other bands differing from the mobility of the germline and Δ8 bands were not related in sequence to the ALL-1-derived mRNAs as shown by Southern blot hybridization (data not shown). None of the patients analyzed in Fig. 4A carried a translocation to the ALL-1 gene or a macroscopic alteration of the ALL-1 gene, as far as being detectable by Southern blot experiments (data not shown). Similar RT-PCR assays were also performed with mRNA from MNCs of healthy donors, and variant PCR products identical in size with the Δ8 product from ALL patients were obtained in all cases (Fig. 4B).

**DISCUSSION**

A Novel Type of Structural Alteration of the ALL-1 Gene Associated with T-ALL. A specific deletion in the breakpoint cluster region of the ALL-1 gene is reported here that represents a third type of genomic alteration associated with hematological neoplasias apart from the extensively described chromosomal translocations (reviewed in Ref. 22) and internal duplications of the ALL-1 gene (40, 41). Interestingly, different structural alterations of the ALL-1 gene and protein appear to be associated with different disease phenotypes. The characteristic high-risk infant, pro-B cell leukemias are predominantly associated with translocations t(4;ll) and t(ll;19), whereas translocation t(9;11) is associated mainly with childhood AML and the internal duplications of the ALL-1 gene without translocations are associated with adult AML (40, 41). Thus, a correspondence between the disease phenotype and the type of structural alteration of the ALL-1 protein is likely to exist but has not yet been proven. At present, it is still a conjecture that the altered ALL-1 proteins are the responsible etiological agents for these leukemias. One possibility is that simple genomic deletions of exon 8 as described here may be associated with various ALLs but not necessarily with the characteristic high-risk pro-B ALLs. Patients A1, A8, and A15 support this argument. They are the only patients in our panel showing this deletion, and all three presented with T-ALL but not with pro-B ALL or AML. The self-fused ALL-1 protein variants may then preferably
SPECIFIC DELETION OF ALL-1 SEQUENCES IN ALL PATIENTS

Fig. 2. Exon 8 is lost on one allele only, and the loss is associated with ALL-A. A, schematic illustration of the deletion-specific PCR assay. The numbering of the exons was according to Gu et al. (3). Primer 8-51 binds only to the intact (germline) allele of the ALL-1 gene. Primers 7-3 × 9-54 produce a short 221-bp amplimer from alleles carrying the deletion (Δ8), whereas primers 7-3 × 8-51 produce a long 421-bp amplimer only from intact (germline) alleles. The long 1855-bp amplimer produced by primer combination 7-3 × 9-54 from intact alleles was not detected under our PCR conditions. B, a representative subset of the analyses performed with biopsy material from ALL, AML, and FL patients and PBLs from healthy donors. Open arrowhead, the specific 221-bp amplimer from the allele carrying the deletion; closed arrowhead, the germline allele. The negative control (neg.), containing all ingredients of the assay except genomic DNA, demonstrates that the amplimers were specifically produced from added human template DNA. Agarose gel was stained with ethidium bromide. C. sequences of the regions from introns 7 (upper) and 8 (lower) participating in the deletion. The cross-over site is underlined. Palindromic sequences (5' TGATCA-3' and 5'-CTCGAG-3') are shaded in gray. The fusion point of introns 7 and 8 was identical in all three patients (A1, A8, and A15), as verified by sequence analysis. The degree of overall sequence identity (11 of 47 nucleotides, 23% identity) of both regions is too low for a homologous recombination event explained by the properties of known recombinases and recombination mechanisms.

A

B

C

generate AML, and to produce a high-risk pro-B-ALL with the characteristic phenotype outlined above, the additional chimeric proteins generated from the breakpoint regions of the reciprocal derivative chromosomes (der4 and der19), which are always present in the leukemic blasts, may be required. The panel of patients analyzed here is still too small to produce a definitive statistical correlation, and it contained patients with other subtypes of T-ALL that did not show this deletion, but the absence of the deletion in AML and FL samples is conspicuous. A larger number of cases needs to be investigated for a critical test of the hypothesis that the deletion of exon 8 defines a new disease entity, i.e., a new subtype of T-ALLs different from the characteristic high-risk, infant pro-B ALLs.

Rare cases of childhood T-ALL associated with t(11;19) translocations have been reported (14, 46). However, in these cases, the translocation was only mapped at a cytogenetic level, and no direct evidence for a structural alteration of the ALL-1 gene was provided. In one additional case of childhood T-ALL, a complex rearrangement between the ALL-1 and ENL genes was reported (47). An ALL-1/ENL fusion mRNA species was expressed in these cells. This interesting observation suggests a possible extension of our hypothesis; apart from the exon 8 deletion, other structural alterations of the ALL-1 gene may also generate novel T-cell oncogenes.

Mechanism of Deletion of Exon 8. The area around exon 7 is one of the most frequently used sites for de novo translocations to the ALL-1 gene (39, 48), apart from the triple Alu element in intron 6, where about 40% of all reciprocal translocations have been mapped (40, 41, 48). The specific genomic deletion of exon 8 reported here occurred between a palindromic sequence 19 bp downstream of exon 7 (TGATCA, a BclI restriction site) and another palindrome located in the first Alu element of intron 8 (CTCGAG, an Xhol restriction site). The palindromic CTCGAG sequence was not found in the seven other Alu repeats contained in the breakpoint cluster region (39, 48). Illegitimate recombination between both palindromic sequences probably occurred at a C-nucleotide by a yet unknown mechanism, resulting in the specific deletion of a 1634-bp DNA fragment, including exon 8 and surrounding intron sequences on either side. Interestingly, the deletion eliminated precisely the same sequence in all three unrelated patients. It occurred in the narrow break-point cluster region of the ALL-1 gene (10, 49) and, therefore, may have been produced by a mechanism similar or identical to that generating the characteristic translocations. By contrast, the self-fusions of the ALL-1 gene in AML patients have been proposed to be generated by homologous Alu-Alu recombination (40, 41), which if confirmed would be a mechanism different from the one causing the genomic deletions of exon 8.

A Potential Role for Exon 8-derived Amino Acid Sequences in the ALL-1 Protein and Possible Oncogenic Functions of ALL-1 Variant Proteins. Exon 8 specifies part of a predicted zinc finger domain of the ALL-1 protein (3, 4, 39) encoded by exons 8 to 13. The amino acid sequence allows the formation of seven zinc fingers, and DNA binding activity has been anticipated for this domain (3, 39). However, the amino acid sequence of this region may also be folded alternatively to form a set of so-called "PHD-fingers" (50). Six of the seven zinc fingers can be folded into three PHD-fingers, which would confer a potential for protein-protein interactions to this domain.
Consensus PHD-fingers have the amino acid sequence [N'-Cys-(2)-Cys-(n)-Cys-(2)-Cys-(4)-His-(2)-Cys-(n)-Cys-(2)-Cys-C'], where the numbers in parentheses specify the numbers of intervening amino acid residues and can bind two zinc atoms (50). The first putative zinc- or PHD-finger will be missing in the translation products from mRNAs lacking exon 8. Conceivably, this alteration might endow the deletion variant with the ability to bind to the intact ALL-1 protein and interfere with its function in a dominant-negative fashion. A similar mechanism may be applicable to the internal duplication variant of the ALL-1 protein. To provide direct proof for any hypothesis about the functions of wild-type and mutant ALL-1 proteins, it is necessary to introduce the coding sequences for these proteins into hematopoietic progenitor cells and to monitor the effects in a biological assay system, either a cell culture or a transgenic animal system. The reciprocal experiments would also provide partial proof, i.e., elimination of the coding sequences for these protein variants from leukemic cells and demonstration of a partial or complete reversion to the nontransformed phenotype. Such experiments require the introduction of antisense constructs or targeting constructs for homologous recombination and the use of cell culture and animal models. They are technically demanding, and to our knowledge, no experimental results providing direct proof for the mechanisms may only be reached in progenitor cells carrying the genomic deletion, but not in PBLs carrying the alternatively spliced mRNA species in healthy donors. Another explanation needs to be considered. The corresponding protein variants lacking exon 8-derived sequences have not yet been detected and quantitated on the protein level due to the lack of specific reagents. Therefore, the concentrations of the variant mRNA seen in peripheral blood cells may not be proportional to the concentration of the variant protein in progenitor cells. If a critical threshold concentration of this protein were required to achieve transformation in progenitor cells, then these concentrations may only be reached in progenitor cells carrying the genomic deletion, but not in PBLs carrying the alternatively spliced mRNA variant. The specific genomic deletion identified here in biopsy material of three unrelated T-ALL patients may result in the continuous expression of the ALL-1 Δ8 mRNA variant in early progenitor cells, where it is not normally present. Thus, if the corresponding ALL-1 protein variant had a transforming effect specific for early progenitor cells, then the set of data presented here would be free of inconsistencies.

Specific Functions of the Exon 8 Deletion Variant of the ALL-1 Protein in T Cells. The observation that chimeric variants of the ALL-1 protein correlated with pro-B ALL, self-fused variants with AML, and exon 8 deletion variants with T-ALL makes the conclusion almost unavoidable, that the variant ALL-1 proteins are the oncogenic
agents causing these diseases. The evidence is still circumstantial and not direct, but the degree of confidence that can be placed in this conclusion has now reached virtual certainty. Direct proof of this hypothesis still requires the gene transfer experiments described above. The observations reported here do not address the interesting question, how a deletion of exon 8 sequences confers T-lineage specificity and self-fusion myeloid lineage- and chimerization B-lineage specificity. Two other cases of T-ALL associated with an alteration of the ALL-1 gene have been reported, both translocations t(X;11) of the AF-X1 gene to the ALL-1 gene (33, 34). It would be interesting to determine whether, in these cases, exon 8 sequences were also lacking in the chimeric proteins derived from the der11 chromosomes or whether the T-lineage specificity was due to a contribution from the chimeric protein derived from the derX chromosomes, or a combination of both.

Drosophila trx mRNA is also alternatively spliced in a cell type- and stage-specific manner during development (51). However, the alternative splicing pattern documented in this report affects only the first four exons of the Drosophila trx gene, for which there is no highly conserved sequence in the ALL-1 mRNA. Alternative splicing within the trx zinc finger region (amino acids 1269 to 1841) has been demonstrated thus far; therefore, alternative splicing of exon 8 in the human ALL-1 mRNA has no direct counterpart in the Drosophila trx system.

Practical Value. The genomic deletion of exon 8 provides investigators with a useful marker for diagnostic purposes and for monitoring the success of therapy and the progress of minimal residual disease in ALL patients that carried this deletion.

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