RAPID COMMUNICATION

Unexpected Heterogeneity in E2A/PBX1 Fusion Messenger RNA Detected by the Polymerase Chain Reaction in Pediatric Patients With Acute Lymphoblastic Leukemia

By Shai Izraeli, Heinrich Kovar, Helmut Gadner, and Thomas Lion

The t(1;19)(q23;p13) is the most common recurring chromosomal translocation in childhood acute lymphoblastic leukemia (ALL) and has been associated with adverse prognosis. It involves the rearrangement of two genes, PBX1 and E2A, resulting in the production of transforming chimeric DNA-binding proteins. In all previous reports in which the presence of a chimeric transcript was described, the fusion point between the coding sequences of E2A and PBX1 was found to be constant at the RNA level. We have used RNA-based polymerase chain reaction (PCR) for the detection of E2A/PBX1 messenger RNAs (mRNAs) in children with ALL at the time of diagnosis. Of 21 patients exhibiting this rearrangement, 3 (14%) expressed a variant E2A/PBX1 transcript in addition to the expected one. The relative amounts of the two chimeric mRNAs varied between the patients, but remained constant in the same patient during different stages of the disease. Sequence analysis showed an identical insertion of 27 bp at the E2A/PBX1 junction of the variant RNA species, the translation of which would result in the replacement of Val478 by 10 amino acids. The inserted sequence has not been detected in any other human transcript besides the variant E2A/PBX1 RNA species and probably represents a splicing variant of the chimeric RNA. We conclude that a subset of pediatric patients with ALL that carry the E2A/PBX1 rearrangement express two types of the chimeric mRNA. The biologic significance of this additional E2A/PBX1 transcript is discussed.

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MATERIALS AND METHODS

Patients and cell lines. The patients described are part of a group of 21 pediatric patients with ALL carrying the E2A/PBX1 rearrangement as identified by RNA-based PCR analysis of leukemic cells at the time of diagnosis. All patients were treated according to the Berlin-Frankfurt-Münster (BFM) protocols for ALL.1,11 The cell line 697, which carries the t(1;19) translocation, was obtained from Dr H. Drexler (Braunschweig, Germany). SUP-B15 is a Ph1 chromosome-positive ALL cell line obtained from Dr S. Smith (University of Chicago, Chicago, IL). The neuroblastoma cell line STA-NB-2 was established at the Children's Cancer Research Institute (CCRI) and the neuroblastoma cell lines VI86 and LA-N-1 were obtained from Dr O. Majdic (Institute of Immunology, Vienna, Austria) and from Dr R.C. Seeger (Department of Pediatrics, UCLA, Los Angeles, CA).

PCR. RNA extraction and reversed PCR were performed as described previously.10 To verify the specificity of amplified products and to increase the sensitivity, 3 μL of a 1:1,000 dilution of the first round product was subjected to a second round of nested PCR for 35 cycles. The quality of the cDNA and the validity of the negative results were assessed by parallel amplification of the ubiquitously expressed E2A mRNA. Strict precautions against contamination were undertaken.14 Negative and positive controls for preparation of cDNA and for PCR were included in each experiment.

From the Children’s Cancer Research Institute, St Anna Children’s Hospital, Vienna, Austria.

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Address reprint requests to Heinrich Kovar, PhD, CCRI, St Anna Kinderspital, Kinderspitalgasse 6, A-1090 Wien, Austria.

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were included throughout RNA extraction, reverse transcription, and PCR. PCR of every positive case was repeated with new cDNA and, if available, with a different RNA preparation from another cell specimen of the same patient. PCR products were separated in 3% agarose gels and visualized by staining with ethidium bromide.

Hybridization. Northern and Southern blot hybridizations to radiolabeled oligonucleotides were performed essentially as described previously.15

Sequencing and SSCP analysis. Direct sequencing of PCR products was performed essentially as reported previously.16 Confirmation of the sequence identity of PCR products was performed by analysis of single strand conformation polymorphisms (SSCP)17 as described by Orita et al.18

RESULTS

Identification of E2A/PBX1 type Ia RNA. All 21 patients in whom E2A/PBX1 fusion mRNA was detected by PCR exhibited amplification products of the expected size (Fig 1, type I; Fig 2). The correspondence of these PCR products with the published E2A/PBX1 sequence4 was verified by direct sequencing in two cases and by SSCP analysis in the remaining patients (data not shown). Unexpectedly, in three patients, an additional, slightly longer transcript was amplified by PCR (Fig 1, type Ia). Coamplification of two different E2A/PBX1 fragments in the same PCR reaction provides a basis for quantitative estimation of the relative amounts of both mRNA species in the cells analyzed.19

The proportions between the common and the variant E2A/PBX1 transcripts amplified showed interindividual differences. Type Ia transcript was the predominant PCR product in patient B, whereas type I was much more abundant in patient A (Fig 1A). In the third patient, both transcripts were present in equal amounts (data not shown). However, within the same patient, the ratio between the quantities of the two transcripts was constant in repeated experiments with different cDNA preparations. Moreover, in one patient, PCR analyses of bone marrow specimens from the time of relapse (Fig 1A, lane A2) showed the same relative amounts of the two alternative transcripts as observed in a specimen collected at diagnosis (Fig 1A, lane A1).

Sequencing of the type Ia fragment showed identical insertions of 27 bp at the E2A/PBX1 junction in all three patients (Fig 1B). Amplified E2A/PBX1 products from all 21 patients investigated were hybridized to probes specific for the variant insert of the type Ia transcript. Positive signals were obtained only in specimens from the three patients, in whom an additional band was already seen after electrophoresis of the PCR products in ethidium bromide-stained gels (data not shown). As shown in Fig 2, no transcripts containing sequences homologous to the 27-bp insert were identified in ALL cell lines containing either the E2A/PBX1 (cell lines 697, lanes 3, 4, and 6) or the BCR/ABL rearrangement (SUP-B15, lanes 1, 2, and 5) or in neuroblastoma cell lines expressing a normal PBX1 transcript (STA-NB-2, LA-N-1, and VI 856, lanes 8, 9, and 10, respectively), which is slightly smaller than the rearranged one (compare lanes 11 and 12).

Origin of the insert in E2A/PBX1 type Ia mRNA. Comparison of the insert in the E2A/PBX1 transcript type Ia to sequence data bases showed no significant homology to any known sequence, including the E2A coding sequence.6 In addition, a 146-bp fragment amplified from a normal PBX1 cDNA and located upstream of the rearrangement point in E2A/PBX1 chimeric RNA does not include the type Ia-specific 27-bp segment (data not shown). The insert was used as an oligonucleotide probe for hybridization to Southern blots of genomic DNA from cells expressing or lacking the variant E2A/PBX1 mRNA. In all instances, the probe hybridized to at least 8 different fragments generated by digestion with various restriction enzymes. At least some of these fragments originated from chromosomes other than 1 or 19, as indicated by analysis of mouse-human hybrid cell lines containing small numbers of defined human chromosomes. Northern blot hybridization of total cellular RNA to a variant-specific sense oligonucleotide showed that sequences homologous to the 27-bp insert are present in some expressed genes in inverted orientation (data not shown). Similarly, a number of different restriction fragments derived from PBX1-related sequences hybridized to a PBX1-specific oligonucleotide that flanks the rearrangement site in E2A/PBX1 transcripts.19

Amplification of genomic DNA with either E2A or PBX1 upstream and downstream primers flanking the rearrangement site did not yield any PCR products hybridizing to the variant type Ia insert. Additional DNA- and RNA-based PCR
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Fig 2. Detection of variant E2A/PBX1 transcripts by Northern blot analysis. Total (lanes 2, 4, 7, and 11) or poly A selected (lanes 1, 3, 5, 6, 8, 9, 10, and 12) RNA was hybridized either to an oligonucleotide homologous to the type Ia insert (lanes 1 through 10) or to an 88-bp PBX1-specific probe generated by PCR amplification (lanes 11 and 12). Hybridization to a human β-actin probe is shown as a control. Lanes 1, 2, and 5, ALL cell line SUP-B15; lanes 3, 4, 6, and 11, ALL cell line 697; lane 7, ALL patient B (compare Fig 1); lanes 8 and 12, neuroblastoma cell line STA-NB-2; lane 9, neuroblastoma cell line LA-N-1; lane 10, neuroblastoma cell line VI 856. The arrows point to the chimeric E2A/PBX1 transcripts type I (lane 11) and type Ia (lane 7), respectively.

experiments with different combinations of oligonucleotides derived from the type Ia insert and either E2A or PBX1 upstream and downstream sequences, respectively, failed to clarify the origin of the inserted sequence in the type Ia transcript (data not shown).

DISCUSSION

In this report, we show that a subset of pediatric ALL patients carrying the E2A/PBX1 rearrangement express a hitherto unknown variant E2A/PBX1 fusion mRNA. In our series, the novel RNA species was detected in 3 of 21 patients investigated. This variant chimeric mRNA, which we termed “type Ia” transcript, contained an insert of 27 bases at the E2A/PBX1 junction. In a previous study, PCR analysis in a larger series of ALL patients displaying PCR-detectable chimeric E2A/PBX1 RNA showed only one type of E2A/PBX1 transcript. Therefore, the detection of an additional E2A/PBX1 mRNA species was surprising. In the study conducted by Hunger et al, the PCR products were separated in 1.3% agarose gels. Therefore, the presence of the type Ia transcript in some of these patients cannot be completely excluded because an additional amplification product differing in size by 27 bp may have escaped the detection. Moreover, the oligonucleotide probe used in the previous study for the identification of the specific E2A/PBX1 sequences would have hybridized only to the PCR products resulting from transcript type I, but not type Ia. It is possible, therefore, that reexamination of these cases would result in the identification of patients expressing the type Ia mRNA species. The ALL patients in whom both types of the chimeric message were detected expressed the two E2A/PBX1 species at different proportions (Fig 1A). In the same individual patient, however, the ratio remained constant in different samples analyzed during the course of the disease. We suggest, therefore, that type I and type Ia E2A/PBX1 fusion mRNAs are splicing variants of the same rearrangement that are coexpressed in the same malignant clone. When regarding ALL as a clonal disease, the coexistence of two leukemic clones, in each of which a different type of E2A/PBX1 rearrangement is expressed, would be a less likely interpretation of our results.

Computer-aided theoretical secondary structure analysis of the type Ia chimeric protein by the method of Chou and Fasman suggested that the insertion does not significantly alter the protein structure. Thus, the functional activity of the chimeric protein is probably not impaired. Several lines of evidence suggest that the presence of type Ia E2A/PBX1 transcript may have biologic and clinical importance. Only a subset of patients expresses this variant mRNA and, as shown in patient B, type Ia may be the predominant transcript. Moreover, the additional sequence is inserted in-frame. The chimeric protein encoded may therefore maintain the E2A and PBX1 domains found in type I, but contains a replacement of Val 478 by 10 amino acids at the junction site.

Alternative splicing of chimeric RNAs seems to be common in cells carrying chromosomal translocations. In
the (t(9;22)(q34;q11), a translocation characteristic for chronic myeloid leukemia and a subset of ALL, ABL exon II alternatively splices to one of two adjacent BCR exons. Similarly, in patients with acute promyelocytic leukemia, splicing variants of the PML/RAR fusion gene associated with the translocation t(15;17)(q22;q11-21) have been recently described. Kamps et al reported the detection of splicing variants of the PML/RAR fusion gene associated with the t(9;22)(q34;q11), a translocation characteristic for the t(1;19)(q23;p13). A Pediatric Oncology Group Study. Blood 76:117, 1990.

Abbreviation: CCR1 complete clinical remission 1.

*Current status 13 to 78 months after diagnosis. Immunologic subtypes were classified as follows: cALL: CD19+, TdT+, HLADR+, CD10+, clg-; ALL: CD19+, TdT+, HLADR+, CD10+, clg not determined; pre-B-ALL: CD19+, TdT+, HLADR+, CD10+, clg*. The letters A, B, and C (see Fig 1A) designate patients displaying both transcripts (I + Ia). Data from the 18 patients expressing only type I E2A/PBX1 mRNA are presented as a summary. The figures in parentheses indicate the number of patients.

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