Expression of the ABL-BCR Fusion Gene in Philadelphia-Positive Acute Lymphoblastic Leukemia

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We have previously shown that the chimeric gene ABL-BCR, formed on the derivative chromosome 9q+ as a result of the t(9;22) translocation, is transcriptionally active in 65% of chronic myeloid leukemia patients. We have now used the same technique, reverse transcription/polymerase chain reaction amplification of ABL-BCR transcripts, to study nine patients with Philadelphia (Ph) chromosome-positive acute lymphoblastic leukemia (ALL); seven expressed the P190 and two the P210 type of BCR-ABL fusion protein. All seven patients with P190 had ABL-BCR transcripts containing a junction between ABL exon 1b and BCR exon 2 (1b-e2); in two cases, ABL-BCR transcripts with the 1a-e2 junction type were also present. Of the two P210 ALL patients, one had a 1b-b4 ABL-BCR transcript and the other showed no detectable ABL-BCR expression. Although the BCR-ABL gene is probably fundamental in the pathogenesis of the Ph+ leukemias, differential expression of the ABL-BCR gene could contribute to the biologic heterogeneity of the disease.

MATERIALS AND METHODS

Patients and cell lines. Nine consecutive adult patients with Ph+ ALL were identified from a larger series of acute leukemias on the basis of cytogenetics and molecular criteria (Tuszynski et al, manuscript submitted). Eight patients were studied at diagnosis and one at relapse after chemotherapy. The leukemic cells were CALLA+ in 8 cases, but blast cells from one patient had a "null" phenotype. The mean absolute blast count in this series was 48.4 × 10⁹/L, with a range of 1.9 to 138.6 × 10⁹/L. Seven patients expressed P190CR-ABL protein and two expressed P210CR-ABL as detected by in vitro kinase assays. Two Ph+ CML patients with known BCR-ABL and ABL-BCR transcript types were also included for comparison with the ALL samples.

SD1 is a lymphoblastoid cell line established by Epstein-Barr virus (EBV) immortalization of Ph+ P190 ALL cells from one of the patients studied in the present series. The HL60 promyelocytic cell line was used as a negative control for BCR-ABL and ABL-BCR expression.

Polymerase chain reaction (PCR) amplification. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density-gradient centrifugation and used for RNA extraction. Amplification of specific sequences on the ABL, BCR, BCR-ABL, and ABL-BCR genes was performed by reverse transcription (RT) of oligo-dT-primed cDNA, followed by PCR (RT/PCR) exactly as described previously.

The synthetic oligonucleotides used for PCR amplification were: the primers PAd, Ab+, Jc-, B4-, and G+, described previously; two new BCR primers, BAL+ (in exon 1e) (5')GCAGAAGGAGCGACAGTCCTTCCGGAC(3') and BE2+ (in exon e2) (5')AGGTGATTGTCTCTGACAGGAT(3'); and one new ABL primer, GLY+ (in exon 1b) (5')GGCGAGCCGCTGAGAACAG(3'). The relative positions of primers on BCR and ABL sequences are shown on Fig 1.

Each PCR experiment was repeated at least once and included two negative controls (HL60 cDNA and no cDNA template). Further precautions towards eliminating the possibility of false PCR results were described elsewhere.

Direct sequencing of PCR products was performed by the Taq cycle sequencing method (US Biochemicals, Cleveland, OH).

RESULTS

Expression of the normal ABL and the normal BCR genes were detected in all nine ALL cases and in the SD1 cell line by means of PCR amplification with the PAd+ → Jc- (384 bp), Ab+ ↔ Jc- (462 bp), and B4+ ↔ G+ (1,208 bp) primer pairs.

PCR amplification with primers BAL+ and Jc- showed BCR-ABL transcripts of 463 bp, corresponding to a ela2 junction, in the seven P190CR-ABL samples and in the SD1 cell line (Fig 2A, lanes 2 through 9). The two P210CR-ABL
ALL samples did not yield detectable PCR products with these primers, but showed positive amplifications with primers B1+ and Jc−; one sample exhibited a b3a2 junction type and in the other an exceptionally small BCR-ABL product was detected (Fig 2A, lanes 12 and 13, respectively). Sequencing of the latter showed that the BCR-ABL junction in this sample was between BCR exon b2 and ABL exon 3 (b2a3).

Expression of the reciprocal ABL-BCR fusion gene was investigated by PCR amplification of fragments encompassing ABL exons Ib or Ia, and BCR sequences downstream of exon el, as predicted from the breakpoint sites in ABL and BCR, respectively. By using primers GLY+ and BE2−, PCR products 404-bp long were detected in all seven P190BCR-ABL ALL samples (Fig 2B, lanes 2 through 8), characterizing an be2 junction in these cases. Amplifications spanning ABL-BCR sequences between primers PAA+ and BE2− showed transcripts of the Ia-e2 type in only two of the seven P190 ALL samples. The SD1 cell line exhibited no ABL-BCR expression (Fig 2B, lane 9).

As expected, no ABL-BCR amplification was observed with either primer pair in the two ALL cases with a P210BCR-ABL product, because the breakpoint in these cases occurs in the M-bcr region. Nevertheless, PCR tests with primers used for identifying ABL-BCR message in CML (Ab+ ↔ G− and PAA+ ↔ G−) showed amplification of the Ib-b4 transcript type in one sample, but no ABL-BCR product in the other (Fig 2B, lanes 12 and 13, respectively).

Table 1 summarizes the overall results on BCR-ABL and ABL-BCR expression in this series. These results were further verified by amplification of a larger ABL-BCR fragment in reactions primed with the oligonucleotides Ab+ and G− and PAA+ and -G− (lanes 2 through 16). Molecular weight markers were pEMBL digested with Taq I (lane 1) and a 1-kb DNA ladder (lane 11).
occurs in Ph+ ALL. By means of single-step RT/PCR amplifications, we detected ABL-BCR transcripts with an I-b2 junction in all seven P190<sub>BCR-ABL</sub> patients tested in the present study. Only two of seven samples expressed ABL-BCR messages derived from both ABL first alternative exons Ib and Ia (I-b2 and Ia-e2), suggesting that, as in CML, the frequency of breakpoints between exons Ib and Ia is significantly higher than that between exons Ia and II. On the other hand, formation and expression of the ABL-BCR gene may be more common in P190 ALL (100% in this series) than in CML (70%), although the number of samples we tested is small. If confirmed in a larger series, this finding suggests that events in 30% of CMLs preclude the formation of an intact ABL-BCR gene, such as breakpoints in chromosome 9 upstream of exon Ib or deletions of 3' BCR sequences, are extremely rare in P190 ALL.

The SD1 cell line did not express any ABL-BCR product, although this line carries the 9q+ derivative chromosome. In contrast, the patient's cells from which the SD1 cell line was derived were found to express the ABL-BCR gene. This disparity is not readily explained, but immortalization with EBV could have acted to downregulate ABL-BCR expression.

ABL-BCR expression can also be found in Ph+ ALL of the P210 type, as exemplified by the detection of an Ib-b4 transcript type in one of the two samples tested here. The other P210 ALL studied has an unusual, albeit not unprecedented, BCR-ABL transcript in which ABL exon 3, rather than 2, is fused to BCR exon b2 (b2a3 junction). The reciprocal ABL-BCR message in this case should be longer, with ABL exon 2 joined in phase to BCR exon b3 (a2b3). In practice, no ABL-BCR transcript was detected in this case, despite repeated studies with various primer pairs (data not shown). The mechanism accounting for the absence of ABL-BCR expression in this case could be the same as for the one-third of CML patients who are ABL-BCR negative, as discussed above and elsewhere.

The predicted coding sequences of the ABL-BCR proteins in P190 ALL are in frame, as they are in CML and in P210 ALL, with ABL contributing the first base and BCR the second and third bases of the junction codon. It is interesting that this junction codon is an aspartic acid in all the 6 possible types of ABL-BCR junction observed so far (ie, Ib-b3, Ia-b3, Ib-b4, Ia-b4 in CML and P210 ALL, and I-b2 and Ia-e2 in P190 ALL).

### Table 1. Junction Type of the BCR-ABL and ABL(1b)-BCR Transcripts

<table>
<thead>
<tr>
<th>Cells</th>
<th>BCR-ABL Protein</th>
<th>BCR-ABL mRNA</th>
<th>ABL(1b)-BCR Protein</th>
<th>ABL(1b)-BCR mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 7</td>
<td>P190</td>
<td>Ia-e2</td>
<td>Ia-e2</td>
<td></td>
</tr>
<tr>
<td>n = 1</td>
<td>P210</td>
<td>b3a2</td>
<td>Ia-e2</td>
<td></td>
</tr>
<tr>
<td>n = 1</td>
<td>P210</td>
<td>b2a3</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>SD1 line</td>
<td>P190</td>
<td>Ia-e2</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ND, not detected.

The open reading frame of the ABL-BCR hybrid cDNAs from P190 ALL predicts fusion proteins of 890 (Ib-e2) and 871 (Ia-e2) amino acids, which are more than double the size of the predicted ABL-BCR gene products of CML and P210 ALL. In all cases the ABL-BCR proteins would contain the C-terminal GAP<sup>PP</sup> coding domain of BCR. However, another domain recently identified on the BCR gene with homology to the db1 oncogene, the VAV proto-oncogene and the yeast CDC42<sup>Hs</sup>, would be variably located in the BCR-ABL and the ABL-BCR fusion proteins of P210 and P190 Ph+ leukemias. Thus, breakpoints in M-bcr (CML and P210 ALL) lead to inclusion of the db1-like domain in the BCR-ABL protein, whereas breakpoints in m-bcr (P190 ALL) result in this domain being included in the predicted reciprocal ABL-BCR, instead of the BCR-ABL fusion product. The db1-like domain encodes a GDP-GTP exchange factor for the RAS-like polypeptide CDC42<sup>Hs</sup>, which suggests that BCR may function through the regulation of a RAS-like signal transduction pathway. It has been proposed that the contribution of the exchange factor domain to P210 but not P190 may explain the qualitative biologic differences between these two forms of BCR-ABL protein. Conversely, it is conceivable that the presence or absence of the db1-like domain in the putative reciprocal ABL-BCR protein will dictate its functional relevance.

**ACKNOWLEDGMENT**

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### REFERENCES


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