The ABL-BCR Fusion Gene Is Expressed in Chronic Myeloid Leukemia

By Junia V. Melo, D.E. Gordon, N.C.P. Cross, and J.M. Goldman

Although the BCR-ABL hybrid gene on chromosome 22q− plays a pivotal role in the pathogenesis of chronic myeloid leukemia (CML), little is known of the reciprocal chimeric gene, ABL-BCR, formed on chromosome 9q+. By reverse transcription/polymerase chain reaction amplification (RT/PCR) we have detected ABL-BCR mRNA in cells from 31 of 44 BCR-ABL positive CML patients and 3 of 5 CML cell lines. Of the 34 positive samples, 31 had classical t(9;22) (q34;q11) translocations; in 3 samples there was no Philadelphia (Ph) and/or 9q+ chromosomes. ABL-BCR expression consisted of ABL(lb)-BCR mRNA in 26 patients and of both ABL(lb)-BCR and ABL(la)-BCR mRNA species in 6 patients. The ABL-BCR transcripts encoded one or, more rarely, both of the two potential junctions, designated ABL-b3 and ABL-b4, which differed in size by 75 bp. In 2 patients, the BCR exon b3 was not present in either the BCR-ABL or the corresponding ABL-BCR transcript, whereas in 5 patients exon b3 was present in both transcripts. Direct sequencing of PCR fragments representing the full-length coding sequence of ABL-BCR cDNAs type Ib-b3, la-b3, lb-b4, and la-b4 showed an open reading frame predicted to encode fusion proteins of 370 to 414 amino acids. If an ABL-BCR gene product is produced in CML cells, it may be relevant as a mechanism for deregulating the GTPase activating protein (GAP) function of BCR.

CHRONIC MYELOID leukemia (CML) is characterized cyogenetically by the presence of the Philadelphia (Ph) chromosome, which originates from the reciprocal translocation t(9;22) (q34;q11). In the formation of the Ph chromosome, the bulk of the ABL protooncogene is translocated from chromosome 9 onto the BCR gene in chromosome 22. This gives rise to a novel chimeric BCR-ABL gene, which encodes a 210-Kd (P210) fusion protein with prominent tyrosine kinase activity and transforming ability.

The product of the normal BCR gene is a 160-Kd (P160BCR) cytosolic phosphoprotein, whose physiological role is not clearly defined. It was shown to form cytoplasmic complexes with P210BCR-ABL in Ph-positive CML cells, as well as with a 53-Kd protein of unknown function in both Ph-positive and Ph-negative cell lines.1,2 Sequences encoded by the first exon of BCR are responsible for the P160BCR serine/threonine kinase activity.3 These sequences overlap the src-homology 2 (SH2)-binding regions of the BCR gene that are essential for the activation of the ABL tyrosine kinase and the transforming potential of the chimeric BCR-ABL oncogene.4,5 In its central segment, BCR has some homology to the dbl oncogene and the yeast CDC24 gene.6 The product of the latter is involved in the control of cell division after DNA replication.7 The C-terminus of BCR has recently been shown to have a GTPase-activating protein (GAP) activity for p21ras, a member of the RAS family of small GTP-binding proteins.8

In the t(9;22) translocation, the p21ras GAP domain of BCR is absent from the BCR-ABL chimeric protein. The 3' end of the BCR gene containing the coding sequence for this domain is, in turn, fused to the 5' end of ABL on chromosome 9. The "fate" of the 9q+ chromosome, the other partner in the reciprocal t(9;22), is not known. Whereas most of the presently available data suggest that the P210 fusion protein encoded by the BCR-ABL hybrid gene is involved in the pathogenesis of CML,6,9,10 it is likely that other genetic changes are also necessary for defining the full leukemic phenotype.

The expression of the 5'ABL-3'BCR hybrid gene in this translocation has not so far been studied, but it may have functional consequences if it leads to abnormal activation of BCR-GAP.

We show here that the reciprocal ABL-BCR gene is transcriptionally active in over two thirds of Ph-positive CML patients, and that translation of its cognate chimeric mRNA into an ABL-BCR fusion protein is compatible with its sequence.

MATERIALS AND METHODS

Patients and cell lines. Cells from a total of 44 CML patients were studied: 20 in chronic phase (CP) and 24 in blast crisis (BC). Among the latter, 15 were myeloid BC, 4 lymphoid, 1 mixed myeloid/lymphoid, and 4 of unknown phenotype. Thirty-eight patients showed characteristic Ph and 9q+ chromosomes, 1 had an atypical 22q− without a 9q+, and 5 were Ph-negative. All patients had clonal BCR gene rearrangement by Southern blot analysis.

Five BCR-ABL-positive cell lines were also investigated. These were well characterized lines from patients with CML in blast crisis: K562, KCL-22, KYO-1, BV173,11 and LAMA-S4.12 The HL60 promyelocytic cell line was used as a negative control for BCR-ABL expression in all tests.

Polymerase chain reaction (PCR) amplification. Amplifications of specific sequences on the ABL, BCR, BCR-ABL, and ABL-BCR genes were performed by reverse transcription (RT) of cDNA, followed by PCR (RT/PCR) by standard methods. Briefly, PB white blood cells (WBC) were obtained by dextran sedimentation or by red cell lysis of centrifuged buffy coat preparations.13 The patients' WBC and freshly explanted cells from lines in culture were washed twice in phosphate buffered saline and processed for RNA extraction by the guanidinium thiocyanate/CsCl gradient method.14 The RNA was reverse transcribed into cDNA with Mo-MuLV reverse transcriptase (GIBCO-BRL, Gaithersburg, MD) using random hexamers and, in some samples, oligo-dT primers. PCR amplification of cDNA was performed as described elsewhere.15 Precautions towards eliminating the possibility of false PCR results were based on the recommendations by Kwok and Higuchi.16 In brief: (1) cells, RNA, and cDNA preparations were always handled in a room separate from that specifically dedicated to the analysis of PCR products; (2) different sets of pipettes were dedicated to sample preparation and PCR product
handling, and plugged tips (aerosol resistant) were used in all steps; 
(3) all reagents were prepared under sterile conditions in a laminar-
flow cabinet, and stored as single-use aliquots; and (4) each PCR 
experiment included 6 to 8 test (CML) cDNA samples plus 2 known 
negative controls: BCR-ABL– and ABL-BCR–negative cDNA from 
HL60 cells, and a H2O-blank (ie, no cDNA). In no instance were 
ABL-BCR products detected in either negative control. Furthermore, 
test samples showing no ABL-BCR amplification alongside samples 
given experiment, reinforcing the validity of the positive results. Five 
microliters from each PCR was electrophoresed through ethidium 
bromide stained 1% to 2% agarose mini-gels, visualized, and pho-
tographed under UV light. Samples that showed no ABL-BCR prod-
uct were submitted to a second round of amplification with nested 
primers and 1 µL of the original PCR products as template.

Primers for PCR, Southern hybridization, and sequencing. The 
sequences of the synthetic oligonucleotide primers used in this 
investigation are shown on Table 1. Some of the primers were designed 
to contain natural or forced restriction enzyme sites at their 5' ends 
to facilitate future cloning of the PCR fragments into plasmid or plasmid 
vector.s The size of PCR fragments amplified with each primer pair 
is shown in Table 2.

Southern hybridization. Electrophoresed PCR products from 
some samples were transferred from agarose gels to nylon membranes 
by Southern blotting, and tested for hybridization to a synthetic oli-

gonucleotide probe (primer B3+) 5'-labeled with γ-32P-ATP. Hybrid-

ization and high-stringency washings were carried out at the appro-
priate discriminating temperatures for the oligonucleotide 
as elsewhere.19

Sequencing. Direct sequencing of PCR products was performed 
by the linear amplification sequencing method20 and/or by the Taq 
cycle sequencing method [USB, Cleveland, OH]. PCR products were 
used directly as templates on an estimated basis of 
100 fmol ss-DNA 
per base per primer. Denaturing sequencing gels were prepared, elec-
trophoresed, and autoradiographed by conventional techniques.

RESULTS

BCR-ABL gene. BCR-ABL amplification of cDNA with 
primers B2* and CA3+ yielded fragments 385 and 465 bp

Table 1. Synthetic Oligonucleotide Primers Used in This Study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (G = 3')</th>
<th>Gene Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aa+</td>
<td>TTTGAGAAGTCCCTCGAAG</td>
<td>ABL (la)</td>
</tr>
<tr>
<td>Ab+</td>
<td>CTCTCTGAAAAAGGCTACGATTA</td>
<td>ABL (lb)</td>
</tr>
<tr>
<td>PAa+</td>
<td>taccggcatatTCTGAGATACTCCTG</td>
<td>ABL (la)</td>
</tr>
<tr>
<td>PAb+</td>
<td>LGTCTAGaattCTGACACTCTG</td>
<td>ABL (lb)</td>
</tr>
<tr>
<td>Jc+</td>
<td>GACGTGTTCCTACAGACTT</td>
<td>ABL (lb)</td>
</tr>
<tr>
<td>CA3+</td>
<td>CGTTGAGCTCGCTGAGTTAGTTGTC</td>
<td>ABL (lb)</td>
</tr>
<tr>
<td>B2*</td>
<td>TCGGAAGCCCTTCCCCTGACAT</td>
<td>ABL (b)</td>
</tr>
<tr>
<td>B3*</td>
<td>TTTAATCTGATTCTCCCTCCCTCCCGGCA</td>
<td>ABL (b)</td>
</tr>
<tr>
<td>B4*</td>
<td>tttccaaATCTCTATCACCCGCTCCTG</td>
<td>ABL (b)</td>
</tr>
<tr>
<td>B7*</td>
<td>GGGATTCTTACACAGGAGGCCACACTCA</td>
<td>BCR (b)</td>
</tr>
<tr>
<td>C4*</td>
<td>attagaTCTAGACGCTTACTGTTGTC</td>
<td>BCR (b)</td>
</tr>
<tr>
<td>Bc*</td>
<td>tcagctgatCTAGTCTGATCTAGCTGAT</td>
<td>BCR (b)</td>
</tr>
<tr>
<td>C5+</td>
<td>ataggatGGTCCTGAACTGCTGCTGAA</td>
<td>BCR (b)</td>
</tr>
<tr>
<td>G*</td>
<td>gctgctgatTCCACCTGCAACACTA</td>
<td>BCR (c)</td>
</tr>
<tr>
<td>PB*</td>
<td>TCAGAAGAGTCCTGCCCTGACGAT</td>
<td>BCR (3'end)</td>
</tr>
</tbody>
</table>

*Lower case indicates nucleotides that are not homologous to the 
cDNA sequence, but incorporate restriction enzyme recognition sites.

Table 2. PCR Amplifications of ABL, BCR, BCR-ABL, 
and ABL-BCR cDNA

<table>
<thead>
<tr>
<th>Primers</th>
<th>Gene</th>
<th>PCR Product (length in bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aa+</td>
<td>ABL (from la)</td>
<td>374</td>
</tr>
<tr>
<td>Ab+</td>
<td>ABL (from lb)</td>
<td>462</td>
</tr>
<tr>
<td>B1*</td>
<td>BCR</td>
<td>1,208</td>
</tr>
<tr>
<td>B2*</td>
<td>BCR-ABL</td>
<td>385 (b2a2)</td>
</tr>
<tr>
<td>PAa+</td>
<td>ABL (la)-BCR</td>
<td>1,072 (la-b4)</td>
</tr>
<tr>
<td>PAb+</td>
<td>ABL (lb)-BCR</td>
<td>1,147 (lb-b3)</td>
</tr>
<tr>
<td>PAa+</td>
<td>PB-</td>
<td>1,223 (lb-b3)</td>
</tr>
<tr>
<td>PAb+</td>
<td>ABL (la)-BCR</td>
<td>1,316 (lb-b4)</td>
</tr>
<tr>
<td>PAa+</td>
<td>PB-</td>
<td>1,391 (lb-b3)</td>
</tr>
<tr>
<td>PAb+</td>
<td>B4*</td>
<td>189 (lb-b4)</td>
</tr>
<tr>
<td>PAb+</td>
<td>B4*</td>
<td>264 (lb-b3)</td>
</tr>
<tr>
<td>B4*</td>
<td>BCR (in ABL-BCR)</td>
<td>456</td>
</tr>
<tr>
<td>B7*</td>
<td>BCR (in ABL-BCR)</td>
<td>398</td>
</tr>
<tr>
<td>C4*</td>
<td>BCR (in ABL-BCR)</td>
<td>527</td>
</tr>
</tbody>
</table>

* These were primers used for direct sequencing of overlapping seg-
ments of the template ABL-BCR amplified cDNAs as PCRAs Aa+  PB- 
and PAb+  PB-.

long, representing the b2a2 and b3a2 type transcripts 
respectively (Fig 1A).

The types of BCR-ABL transcript expressed in the 44 pa-

tients and the five CML cell lines were as follows. In the CP 
group, 45% of patients expressed only b2a2 and 50% only 
b3a2 BCR-ABL transcripts. A relatively similar distribution 
for b2a2 and b3a2 patients was observed in the BC group, 
which includes the five cell lines (59% and 38%, respectively).
One CP and one BC patient expressed both b2a2 and b3a2 
transcripts.

Normal ABL and normal BCR genes. Expression of the 
2 major alternative transcripts from the normal ABL allele 
was observed in all the 44 patients. This was shown by RT/ 
PCR amplification of the sequence spanning exon Ia to exon 
III (PCR PAA+  Jc- ) and, likewise, the sequence from exon 
Ib to exon III (PCR PAb+  Jc- ). Of the five cell lines studied, 
only LAMA-84 and BV173 did not express either ABL tran-
script, which agrees with the absence of a normal chromosome 
9 in these lines.14,21

The normal BCR allele was also found to be expressed in 
all patients and cell lines, as assessed by PCR amplification 
of a 1,208-bp long fragment spanning the major breakpoint 
codon cluster region (M-bcr), and extending downstream of exon 
c7.22,25 This fragment includes the BCR-GAP coding region in 
the normal allele.8,23

ABL-BCR gene. The formation of an ABL-BCR fusion 
gene, the reciprocal product of the BCR-ABL translocation, 
should theoretically yield different transcripts, depending on 
the positions of the breakpoints in both ABL and BCR (Fig 
2). If the breakpoint in ABL occurs upstream of exon Ib, no
ABL-BCR transcript is formed. If the breakpoint is between exons Ib and Ia, only transcripts originate from the exon Ib promoter are possible (Ib-BCR); and if between exons Ia and II, two RNA species can be transcribed from the independent promoters in exons Ib and Ia (Ib-BCR and Ia-BCR).

The 49 samples were tested for expression of ABL-BCR transcripts by RT/PCR amplification using sense primers on ABL exons Ib (Ah') and Ia ( PAa'), and anti-sense primers on BCR 3' end (G- or PB-). A total of 34 out of 49 patients (69%), including 2 Ph-negative patients and the patient without a 9q+ chromosome, showed ABL-BCR amplification of the Ib-BCR type and 6 of these 34 also expressed Ia-BCR. No case expressed Ia-BCR alone (Table 3). Likewise, abnormally large ABL-BCR fragments containing both exons Ib and Ia were never found. In 7 of the 34 samples the ABL-BCR products were only detected by nested PCR. In all the others, the level of ABL-BCR transcripts seemed comparable to that of ABL, BCR, and BCR-ABL amplified products, as estimated within the limitations of a standard, nonquantitative PCR assay. Among the 15 ABL-BCR negative samples, 4 (3 patients and the cell line K562) were Ph-negative, although BCR-rearranged and BCR-ABL positive.

Like BCR-ABL, the ABL-BCR transcripts are predicted to vary in length, depending on whether BCR exons b3 or b4 are joined to the 5'ABL. This was confirmed by Ib-BCR products 1,148 bp and/or 1,223 bp long when primers Ab'- (in ABL exon Ib) and G'- (in BCR), representing fragments 1,223 bp (lb-b3) and/or 1,148 bp (lb-b4) long. Lanes 1 to 10 are CML patient samples; lane 11 is the HL60 cell line (negative control). M, DNA molecular weight marker (pEMBL digested with Tvi I).

Therefore, the ABL-BCR expressing cases fell into 3 categories (Table 4): 12 showing lb-b4 junction, 19 with lb-b3 junction, and 3 cases with double transcripts (lb-b4 and lb-b3). In the 6 patients who also showed Ia-BCR transcription, the junction was of Ia-b4 type in 3 and Ia-b3 in 3. The presence or absence of BCR exon b3 in each transcript was confirmed by Southern hybridization of the ABL-BCR fragments with a 26-mer oligonucleotide (primer B3') spanning a sequence specific for exon b3 (Fig 3) and, in some cases, by direct sequencing of the junction region in each PCR fragment.

Overall, the proportion of ABL-BCR-expressing patients who showed only b2a2 (56%), only b3a2 (38%), and both b2a2 and b3a2 (6%) BCR-ABL transcripts matched the pro-
Fig 2. Schematic representation of the ABL, BCR, BCR-ABL, and ABL-BCR genes. Arrows indicate the most frequent regions for breakpoints in the ABL and the BCR genes. The possible ABL-BCR transcripts arising from the different breakpoints in BCR are shown underneath the corresponding BCR-ABL transcripts type b2a2 and b3a2, respectively.

portion of patients in each BCR-ABL category in the whole series. The frequency of ABL-BCR expression within each BCR-ABL major group was 73% and 62% in the b2a2 and the b3a2 groups, respectively.

When the expression of the BCR-ABL and the ABL-BCR fusion genes was compared in each individual sample (Table 5), it was found that, in 27 patients (79%), the junction type in ABL-BCR was exactly reciprocal to the junction in BCR-ABL. Thus, from the 19 b2a2-positive samples, 15 expressed an ABL-b3 type of junction, and 2 expressed both ABL-b3 and ABL-b4 transcripts. Similarly, 8 out of 13 patients with single b3a2 showed ABL-BCR expression of the ABL-b4 type, as expected. However, in 7 samples the junction type in the ABL-BCR transcripts did not correlate with the reciprocal BCR-ABL products: these were 2 b2a2 patients who expressed only ABL-b4 transcripts, and 5 b3a2 patients in whom ABL-b3 type of transcripts were found either alone (4 samples) or together with ABL-b4 transcripts (1 sample). The junction region of BCR-ABL and ABL-BCR products from this group of patients was sequenced (primers PAa+ ↔ B4+) and results confirmed that, in 2 cases, BCR exon b3 was not expressed in either BCR-ABL or ABL-BCR, whereas, in 5 cases, this exon was present in both fusion-gene products. The same result was obtained by Southern hybridization of BCR-ABL and ABL-BCR products with the oligonucleotide probe B3+ (Fig 3). In 5 of the 7 cases it was possible to repeat the BCR-ABL and ABL-BCR amplifications in duplicate blood samples obtained at different times, and/or in duplicate RNA/cDNA preparations from the original blood samples. The initial results were always reproducible. In 2 patients duplicate samples were not available for analysis.

Coding sequence of the ABL-BCR gene. PCR amplified ABL-BCR fragments from cDNA of 2 samples were sequenced in overlapping segments, using 4 pairs of sense and antisense primers (Table 2). The full-length PCR products represented ABL-BCR type Ib-b3 and Ia-b3 from 1 patient, and Ib-b4 and Ia-b4 from another. Each fragment started 60 bp (Ib) or 10 bp (Ia) upstream of ABL's initiation ATG, and ended 86 bp downstream the stop codon for P160BCR.

### Table 3. ABL-BCR Transcripts Found in CML Patients

<table>
<thead>
<tr>
<th>Stage of Disease</th>
<th>None</th>
<th>Ib-BCR</th>
<th>Ib-BCR and Ia-BCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic phase (20)</td>
<td>5 (25%)</td>
<td>13 (65%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Blast crisis (29)*</td>
<td>10 (34%)</td>
<td>15 (52%)†</td>
<td>4 (14%)</td>
</tr>
<tr>
<td>TOTAL (49)</td>
<td>15 (31%)</td>
<td>28 (57%)</td>
<td>6 (12%)</td>
</tr>
</tbody>
</table>

* Including the 5 CML cell lines.
† Including the cell lines KCL-22, KYO-1, and BV173.

### Table 4. Junction Type of the ABL(Ib)-BCR Transcripts in the Positive Cases

<table>
<thead>
<tr>
<th>Stage of Disease</th>
<th>Ib-b3</th>
<th>Ib-b4</th>
<th>Ib-b3 and Ib-b4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic phase (15)</td>
<td>7 (47%)</td>
<td>6 (40%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Blast crisis (19)*</td>
<td>12 (63%)*</td>
<td>6 (32%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>TOTAL (34)</td>
<td>19 (56%)</td>
<td>12 (35%)</td>
<td>3 (9%)</td>
</tr>
</tbody>
</table>

* Including the cell lines KCL-22, KYO-1, and BV173.
In the 4 products, the sequences matched exactly ABL exon lb-b* or la,* which joined in phase BCR exons b3 or b4, maintaining the open reading frame (ORF) up to the normal BCR stop codon. The ORFs of the ABL-BCR hybrid cDNAs code for predicted fusion proteins of 414 amino-acids (AA) (lb-b3), 395 AA (la-b3), 389 AA (lb-b4), and 370 AA (la-b4).

**DISCUSSION**

In Ph-positive CML, the coding sequences of two genes, ABL and BCR, are disrupted as a result of the reciprocal exchange between chromosomes 9 and 22, and two hybrid genes are formed, BCR-ABL (on 22q, Ph) and ABL-BCR (on 9q+). Expression of the BCR-ABL gene was shown in all cases of Ph-positive CML when the sensitive method of RT/PCR amplification was used. However, it was not known whether the reciprocal ABL-BCR hybrid gene is likewise functionally active in this disease. This possibility was raised previously in a single report when a 3.5

<table>
<thead>
<tr>
<th>Table 5. BCR-ABL Transcript Versus ABL(lb)-BCR Transcript</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCR-ABL</strong> (no. of cases)</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>b2a2 (19)</td>
</tr>
<tr>
<td>b3a2 (13)</td>
</tr>
<tr>
<td>b2a2 and b3a2 (2)</td>
</tr>
</tbody>
</table>

* Cases in which the ABL-BCR transcripts are not reciprocal to the BCR-ABL transcripts.

Fig 3. Southern-blot hybridization with oligonucleotide (primer) B3' of (A) BCR-ABL and (B) ABL-BCR PCR products of the same representative samples shown in Fig 1. The oligonucleotide probe sequence is specific for BCR exon b3. Note that in samples 2 and 8 both BCR-ABL and ABL-BCR transcripts hybridize to this probe, indicating the presence of BCR exon b3 in the two hybrid genes. Conversely, neither BCR-ABL nor ABL-BCR transcripts from sample 9 hybridize to the probe, which confirms the absence of exon b3 in both genes.
In the present study, we show that ABL-BCR expression can be detected by RT/PCR in WBC from approximately 70% of CML patients. It is noteworthy that, among the ABL-BCR positive patients, 2 were Ph-negative and 1 had no 9q+ chromosome, showing that, like BCR-ABL, the ABL-BCR gene rearrangement can also take place in the absence of cytogenetic evidence of a t(9;22).

In one third of our cases, no ABL-BCR expression was detected, in spite of repeated PCR tests with several combinations of primers. This group contains 4 of the Ph-negative, BCR-ABL positive samples that may represent complex and not reciprocal chromosome translocations. The remaining 14 samples (26% of the Ph-positive CML) presumably result from translocation breakpoints in chromosome 9 upstream of ABL exon I, or from deletions in BCR sequences 3' to the chromosome 22 breakpoint.

In neither case would an ABL-BCR hybrid message be present.

The higher proportion (82%) of patients expressing only ABL-BCR transcripts of the Ib-BCR type, compared with those (18%) with transcripts initiated from both exons Ib and Ia (Ib-BCR and Ia-BCR) is probably a reflection of the frequency of breakpoints between exons Ib and Ia, and Ia and II. Because the distance between exons Ib and Ia is greater than 200 kb, approximately 10 times that between exons Ia and II (19 kb), the probability of a break occurring within the first large ABL intron might be 10-fold greater than within the second intron. The fact that a single transcript carrying both exons Ib and Ia was never found shows that the normal mechanism of alternative splicing of these two exons is maintained in the ABL-BCR hybrid gene.

The overall similar distribution of ABL-BCR-positive patients among the b2a2 and b3a2 BCR-ABL categories suggests that there is no simple correlation between the breakpoint sites in ABL and in BCR. On the other hand, the structure of the BCR moiety of the BCR-ABL and ABL-BCR transcripts from 7 patients showed unexpected patterns. In 2 of these, neither BCR-ABL nor ABL-BCR transcripts include BCR exon b3. This means that the breakpoint may be in exon b3 itself, or that this exon was included in deletions at the breakpoint site. Another possibility is that b3 was spliced out in the mature mRNA from whichever of the two chimeric primary transcripts that retained it. Precedents for alternative splicing of exon b3 in BCR-ABL are well established in those patients who express both b2a2 and b3a2 transcripts and, in the present study, by the 3 patients with both Ib-b3 and Ib-b4 ABL-BCR.

More surprising are the 5 patients in this study in whom both BCR-ABL and ABL-BCR transcripts contain BCR exon b3. In 3 of these (3 BC), the karyotype showed single Ph and 9q+ chromosomes, in 1 (CP), the 22q—was longer than a typical Ph and there was no 9q+-, and in the fifth (CP), additional i(Ph) chromosomes probably arose from duplication of the original Ph. Therefore in these cases, there is no evidence that the BCR-ABL and the ABL-BCR hybrid genes are not part of the same clone, which implies that BCR exon b3 alone was duplicated in the t(9;22). Whether this b3 duplication took place before or during the translocation is not known. The breakpoint regions of both BCR-ABL and ABL-BCR in these 5 cases are being cloned for further studies.

The nucleotide sequence of the 4 different ABL-BCR hybrid cDNAs found in our series shows that in each case the 5' ABL-3' BCR junction is in phase as expected. Therefore, ABL-BCR fusion proteins of about 390 AA could be translated, the exact size of each varying according to the exon contribution of ABL and BCR to the transcript. The fact that ABL-BCR amplifications were obtained from oligo-t primed cDNAs indicates the presence of poly-(A) on these transcripts and suggests that translatable RNA is produced. Investigations on the presence of such ABL-BCR proteins in cells from patients with CML are in progress.

The significance of ABL-BCR expression in CML is still unclear. It seems unlikely that the hybrid ABL-BCR gene has any primary oncogenic role, because it is not present in at least one third of the CML patients. Furthermore, the fact that ABL-BCR is expressed in CP as well as in BC of CML argues against a role in causing disease progression. On the other hand, ABL-BCR expression could well be associated with specific clinical and/or hematological features in subsets of CML patients, which may reflect prognosis and response to treatment. Data from our present series are still insufficient to address this question.

If a functional ABL-BCR fusion protein is indeed produced, as predicted from the cDNA coding sequences, it would contain a GAP-BCR domain in its C-terminus linked to an N-terminal ABL sequence. Such an arrangement could alter the racGAP function of BCR leading to either constitutive activation or inactivation of the racGAP activity. Moreover, the Ib-BCR-encoded fusion protein, like the ABL type II P145, would have a myristoylation site at its N-terminus and, therefore, unlike p160BCR, it could become associated with the cell membrane. Although mRNA expression from the normal BCR allele can be detected in CML cells, it is not clear whether the level of P160BCR production is the same as in normal leukocytes. Coexpression of an abnormal ABL-BCR gene product could result in competition for the same target protein, rac, and imbalance in the rate of rac activation. The biologic effects of a deregulated BCR are unknown. However, because the rac proteins display relative myeloid specificity and are involved in activation of the NADPH oxidase system of neutrophils, it is tempting to speculate that an ABL-BCR protein with altered GAP activity might have a role in granulocyte functional defects.
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