p190 BCR-ABL mRNA Is Expressed at Low Levels in p210-Positive Chronic Myeloid and Acute Lymphoblastic Leukemias

By Frits van Rhee, Andreas Hochhaus, Feng Lin, Junia V. Melo, John M. Goldman, and Nicholas C.P. Cross

One hundred and forty-three patients with p210 BCR-ABL–positive leukemia were studied for coexpression of p190 BCR-ABL mRNA. p190 mRNA was detected in 14 of 16 (88%) patients with chronic-phase chronic myeloid leukemia (CML) at diagnosis, in 10 of 10 (100%) CML patients in blast crisis, and in 75 of 107 (70%) CML patients receiving interferon-α (IFN-α). p190 mRNA was detected in 14 of 16 patients with the numbers of p190 transcripts determined by competitive PCR in patients with CML were low compared with the numbers of p210 transcripts. The median numbers of p210 and p190 transcripts per unit volume of cDNA in positive samples were 1.0 × 10^6 (range, 15 to 1.4 × 10^6) and 10 (range, 10 to 2.9 × 10^3), respectively. The numbers of p190 and p210 transcripts were significantly correlated in individual samples (r = .65, P < .001). The median number of p210 BCR-ABL transcripts was significantly lower in samples negative for p190 BCR-ABL transcripts than in samples in which p190 BCR-ABL transcripts were identified (3.1 × 10^5 [n = 73] vs. 1.0 × 10^6 [n = 115]; P < .0001). The median ratio of p190 to p210 BCR-ABL mRNA was not significantly different between chronic-phase CML (1.9 × 10^-4) and CML in blast crisis (1.7 × 10^-4). The median ratio in p210 ALL was also low (1.9 × 10^-3) but significantly higher than that of CML. We conclude that p190 BCR-ABL transcripts are frequently present at a low level in p210 BCR-ABL–positive leukemias. p190 mRNA may arise through alternative or splicing and its presence is probably of no pathogenetic significance.

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dATP, dCTP, dGTP, and 30 U/mL Taq polymerase). PCR was performed on an MJ Research Inc (Watertown, MA) programmable heating block by 30 cycles at 96°C for 1 minute, 68°C for 30 seconds, and 72°C for 2 minutes, followed by 10 minutes of extension at 72°C. The first-step product was diluted 200-fold and 1 µL from this reaction was reamplified with 19 µL of second step mix (second step mix = 125 µmol/L Tris, pH 8.3; 625 µmol/L KCl; 2.25 µmol/L MgCl₂; 0.5 µmol/L primers BCR1⁺ + [5' GAAACTCGCAACAGTCCTTCGAC 3'] and p190[5' B-ABL]; 0.25 µmol/L each of dATP, dCTP, dTTP, and dGTP; and 30 U/mL Taq polymerase).

Amplification was performed for 30 cycles of 96°C for 1 minute, 68°C for 30 seconds, and 72°C for 2 minutes, followed by 10 minutes of extension at 72°C. After second-step PCR, the e1a2 transcript and plasmid p190-C5 yielded bands of 179 and 313 bp, respectively (Fig 2). The point at which the competitor and BCR-ABL bands were of equal fluorescence intensity was determined by densitometry (UVP Ltd, Cambridge, UK) and the results were expressed as p190 BCR-ABL transcript numbers detected in 2.5 µL of cDNA. Four samples from patients with p190 ALL were quantified with the new assay and compared with a standard competitive PCR assay for p190 BCR-ABL mRNA. The results of both assays were concordant.

PCR assays to detect other BCR-ABL fusions mRNAs. CML cDNAs were amplified by nested PCR using primers in BCR exon 1 and ABL exon 11. First-step PCR was with primers BCR-B and ABL11-B (5' AGCGCTGAAACAGTTGCTT 3'); second-step PCR was with primers BCR1⁺ and ABL11-A (5' GAGAAGGCGTTCATCTTTGATC 3'). PCR products were gel-purified, directly sequenced by thermal cycle sequencing with fluorescent dye-terminators, and analyzed on an ABI 373A sequencer. In a second set of experiments, CML cDNAs were amplified by nested PCR with primers in BCR exons 5 or 6 and ABL exon 3. First-step PCR was with primers E5⁺ (5' GAAATGCGTGAAGTCTGCTT 3') and ABL3⁻; second-step PCR was with primers E5/6⁺ (5' GAAATCTCAGGAAAGCTAGGAG 3') and CA3⁻. Southern blots of PCR products were probed with an end-labeled oligonucleotide specific for an e6a2 BCR-ABL fusion (5' CTCTGGAAAAAGGCCCTTC 3').

Statistical analysis. The number of p190 BCR-ABL transcripts present in 2.5 µL cDNA was divided by the number of p210 BCR-ABL transcripts and the result was expressed as a ratio. Comparisons of p190/p210 BCR-ABL ratios were made using the nonparametric Mann-Whitney test, the χ² test, and Spearman’s rank correlation coefficient. For statistical purposes, transcript numbers of less than 10 were assigned a value of 10.

RESULTS

Qualitative RT-PCR. All leukemia samples (n = 188) were positive for p210 BCR-ABL mRNA, p190 BCR-ABL

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**Fig 2.** Quantification of p190 and p210 BCR-ABL transcripts in a chronic-phase CML sample. Fixed amounts of cDNA were coamplified with known numbers of molecules of linearized competitor plasmid using primers specific for either p190 or p210 BCR-ABL mRNA. The point at which the competitor and sample BCR-ABL bands are of equal fluorescence intensity is between 30 and 100 competitor molecules added for p190 and approximately 3 × 10⁶ competitor molecules for p210. Negative control reactions show no amplification products. The specific p190 primers amplify BCR-ABL from a 10⁻⁴ dilution of the p190 BCR-ABL-positive cell line SD1 but do not amplify BCR-ABL from 10⁶ molecules of the p210 competitor construct. M, pEMBL/Taq1 markers.
mRNA was also detected in 14 of 16 (88%) patients with chronic-phase CML at diagnosis, in 75 of 107 (70%) CML patients receiving IFN-α, in 10 of 10 (100%) CML patients in blast crisis, and in 10 of 10 (100%) patients with p210 BCR-ABL-positve ALL (Table 1). Neither p190 nor p210 BCR-ABL transcripts were detected in normal healthy adults.

**Table 1. Expression of p190 BCR-ABL mRNA in p210 CML at Diagnosis, CML on Interferon, CML in Blast Crisis, and p210 ALL**

<table>
<thead>
<tr>
<th>Type and Stage of Leukemia</th>
<th>No. of p190-Positive Patients/Total No. Studied (%)</th>
<th>No. of p190-Positive Samples/Total No. Studied (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML diagnosis</td>
<td>14/16 (88)</td>
<td>14/16 (88)</td>
</tr>
<tr>
<td>CML on IFN-α</td>
<td>75/107 (70)</td>
<td>76/146 (52)</td>
</tr>
<tr>
<td>CML-blast crisis</td>
<td>10/10 (100)</td>
<td>11/11 (100)</td>
</tr>
<tr>
<td>ALL</td>
<td>10/10 (100)</td>
<td>14/15 (93)</td>
</tr>
</tbody>
</table>

**Correlation of p190 BCR-ABL and p210 BCR-ABL mRNA.** There was a significant correlation between the number of p190 and p210 transcripts in all 188 samples (Spearman’s rank correlation coefficient r = .65, P < .001; Fig 3). The median number of p210 transcripts detected in 73 samples negative for p190 BCR-ABL mRNA was significantly lower than the median transcript number detected in 115 samples that tested positive for p190 BCR-ABL mRNA (3.1 × 10^3; range, 10 to 4.5 × 10^3 vs 1.0 × 10^6; range, 15 to 1.4 × 10^6; P < .0001). In 55 samples with p190 transcript levels at the borderline of detection (<10), the median number of p210 transcripts was 2.1 × 10^4 (range, 15 to 9.1 × 10^4). This was significantly higher than the number of p210 transcripts in the p190-negative group (P = .0003), but was significantly lower than the median number of p210 transcripts (1.8 × 10^5; range, 2.9 × 10^5 to 1.4 × 10^6) detected in the 60 samples in which more than 10 p190 transcripts were detected (Fig 4).

The median number of p190 transcripts in all positive samples was 10 (range, 10 to 2.9 × 10^5). The median number of p210 transcripts in all 188 samples was 2.7 × 10^6 (range, 10 to 1.4 × 10^9). Eighty-two of 94 samples with more than 2.7 × 10^6 p210 transcripts were positive for p190 BCR-ABL mRNA. In contrast, only 34 of 94 samples with less than 2.7 × 10^6 p210 transcripts were positive for p190 BCR-ABL mRNA (χ² test, P < .0001).

**Ratio of p190/p210 BCR-ABL mRNAs in the different patient groups.** A comparison was made of the p190/p210 ratio in the 4 different patient groups. There was no significant difference in the median p190/p210 ratio in CML at diagnosis (1.9 × 10^-4; range, 3.0 × 10^-5 to 7.4 × 10^-3; 2 samples tested negative), chronic-phase CML on IFN-α (2.5 × 10^-3; range, 1.9 × 10^-3 to 7.9 × 10^-3; 73 samples tested negative), or CML in blast crisis (1.7 × 10^-4; range, 4.3 × 10^-3 to 6.6 × 10^-4). The median p190/p210 ratio in Ph-positive ALL was 1.9 × 10^-3 (range, 1.3 × 10^-5 to 2.9 × 10^-3; 1 sample tested negative). This was significantly higher compared with the median ratios in CML at diagnosis (P = .04), CML in blast crisis (P = .04), or the IFN-α group (P = .001).

Of the 21 patients who were in complete cytogenetic remission on IFN-α, 5 tested positive for p190 mRNA. These 5 patients all had less than 10 p190 transcripts and between
15 and 1,600 p210 transcripts per 2.5 μL cDNA. The ratio of p190/p210 in these samples appears to be relatively high, but, overall, we did not find any evidence that IFN-α selectively reduced the numbers of p210 transcripts.

The p210 BCR-ABL−positive cell lines K562, KYO1, and KCL22 were all positive for e1a2 transcripts. The p190/p210 ratios in the cell lines were 1.6 × 10^{-4}, 6.4 × 10^{-4}, and 2.1 × 10^{-4}, respectively.

**Search for alternative BCR-ABL fusion mRNAs.** CML cDNAs (n = 20) were amplified by nested PCR using primers in BCR exon 1 and ABL exon 11. Because of the large size of the anticipated p210 and p190 PCR products (approximately 3.3 kb and 1.9 kb, respectively), we reasoned that these amplification reactions would select for shorter alternatively spliced BCR-ABL fusions if they were present. Multiple PCR products smaller than 1.9 kb were generated in most of the samples and 12 of these were sequenced. One product consisted of an intron sequence upstream of ABL exon 11 and may have arisen by mispriming from contaminating genomic DNA. Ten products were fusions of BCR and ABL, but in each case the breakpoints were within exons rather than at exon/intron boundaries. It is likely that these were PCR artifacts arising from partial hybridization of single-stranded ABL and BCR molecules followed by exponential amplification. One product had a precise fusion between BCR exon 1 and ABL exon 8. Such a fusion is unlikely to have arisen as a PCR artifact and probably resulted from a genuine alternative splicing event.

In a second experiment, CML cDNAs (n = 25) and eDNA from a CML patient expressing a rare variant e6a2 BCR-ABL fusion21 were amplified by nested PCR using primers in BCR exon 5 or 6 and ABL exon 3. Products of the expected size for p210 mRNA (b3a2, 1.2 kb; b2a2, 1.1 kb) were generated in all 25 samples; a product of 353 bp was generated from the e6a2 patient. To test the possibility that low-level alternatively spliced e6a2 BCR-ABL transcripts were also amplified in other patients, Southern blots of PCR products were probed with an oligonucleotide probe specific for this junction. E6a2 transcripts were detected only in the patient known to express this fusion mRNA.

**DISCUSSION**

A close relationship has been described between the molecular junction of the fusion gene BCR-ABL and the phenotype of the leukemia. Expression of p190 BCR-ABL mRNA is generally considered to be confined to patients with acute lymphoid or more rarely myeloid leukemias, whereas p210 BCR-ABL mRNA is the hallmark of CML. We report here that p190 BCR-ABL mRNA is frequently detectable in p210 BCR-ABL−positive leukemias. This situation is distinct from that of ALL and the rare cases of CML with a breakpoint in the m-bcr, in which only p190 BCR-ABL mRNA and fusion protein are found.10,11,12,24

In contrast to the study published by Biernaux et al,25 we failed to detect BCR-ABL transcripts in healthy adults. One possible explanation for this discrepancy is that Biernaux et al25 took steps to increase the sensitivity of their RT-PCR assay to 10^{-8}, i.e., approximately 100 times more sensitive than the assay we use.18

There have been sporadic reports in the literature of p190 BCR-ABL mRNA in CML and p210 ALL, and it has been suggested that coexpression of p190 BCR-ABL mRNA is a pathway of progression of CML to blastic phase.26–29 However, we found that transcription of p190 BCR-ABL mRNA is not restricted to patients with acute leukemia or CML in blast crisis, but also occurs in CML at diagnosis and in 70% of CML in chronic phase. It seems unlikely that coexpression of p190 RNA in p210 CML is an important cause of disease progression because the ratio of p190/p210 in CML in blast crisis was similar to the ratios found at diagnosis and in chronic phase.

Quantitation by specific competitive PCR assays showed that the median number of p190 BCR-ABL transcripts in CML was approximately 4 orders of magnitude lower compared with p210 BCR-ABL mRNA. We have previously used competitive PCR to estimate that CML cells express an average of 5 to 10 copies of p210 BCR-ABL mRNA per cell.29 The level of p190 BCR-ABL mRNA in CML suggests that only a single p190 mRNA transcript is detectable per 1,000 cells. This very low level is probably not of pathogenic significance. It is of interest to note that the p190/p210 BCR-ABL ratio in ALL was 1 log higher compared with patients with CML. This raises the possibility that increased expression of p190 BCR-ABL mRNA might confer an acute leukemia phenotype to ALL patients with the p210 BCR-ABL chimeric gene. However, the number of p190 transcripts in these patients is still very low and represents approximately only a single transcript per 100 cells.

The coexpression of p190 BCR-ABL mRNA in patients with p210 CML could perhaps be explained by the acquisition of an additional Ph chromosome with a breakpoint in m-bcr or by the deletion of part of the BCR gene in a subclone. However, the presence of p190 BCR-ABL mRNA in a large proportion of CML patients, the correlation of the number of p190 transcripts to p210 transcripts, and the presence of p190 mRNA in p210-positive CML cell lines suggest that alternative or missplicing of the BCR-ABL primary transcript may be the mechanism. If this is indeed the case, one might have expected to find other low-level alternatively spliced BCR-ABL transcripts. However, apart from a single instance, we have failed to find any evidence of other BCR-ABL fusions. It is possible that splicing of BCR exon 1 and ABL exon 2 is in some way topologically favored. The first introns of both ABL and BCR are very large and the primary transcripts may assume secondary structures that result in the juxtaposition of relevant exons.29

The low numbers of p190 BCR-ABL transcripts present in p210 CML would not be detectable in single-step PCR assays. This may explain why the frequent presence of p190 BCR-ABL mRNA has not been reported previously. Whereas p190 and p210 BCR-ABL proteins can be detected by Western blotting, this technique is not sensitive enough to detect the very low levels of p190 BCR-ABL protein that we would predict to be present in p210-positive samples.30 Dual expression of p190 and p210 BCR-ABL mRNA has no implications for the study of residual disease in CML. However, ALL patients who are positive for p190 BCR-ABL mRNA when first studied in remission should also be
tested for the p210 transcript to exclude or confirm p210 ALL.

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