CASE REPORT

Philadelphia Chromosome-Positive Chronic Myeloid Leukemia Expressing p190BCR-ABL

Akimichi Ohsaka, Shigeo Shiina*, Masaru Kobayashi**, Hideyuki Kudo** and Ryuji Kawaguchi**

Abstract

We describe a case of Philadelphia chromosome-positive chronic myeloid leukemia (Ph-positive CML) expressing p190BCR-ABL. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of bone marrow cells showed a 472-bp band using primers specific for the p190BCR-ABL but not p210BCR-ABL transcript. Sequencing analysis revealed that the PCR product was derived from the fusion between BCR exon e1 and ABL exon a2 (ela2). CML expressing p190BCR-ABL is relatively rare. In a review of the literature, it may be grouped into 2 categories; approximately half of the patients exhibited prominent monocytosis and intermediate hematological phenotype between CML and chronic myelomonocytic leukemia, and the remaining patients showed no monocytosis. (Internal Medicine 41: 1183-1187, 2002)

Key words: minor breakpoint cluster region, ela2 transcript, acute lymphoblastic leukemia, chronic myelomonocytic leukemia

Introduction

Chronic myeloid leukemia (CML) is characterized by the consistent involvement of the Philadelphia chromosome (Ph), which is derived from a reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11). It is found in up to 95% of CML patients and in 20 to 30% of adults with acute lymphoblastic leukemia (ALL) (1). This translocation results in the fusion of the 3' part of the ABL gene on chromosome 9 with the 5' part of the BCR gene on chromosome 22. The breakpoints in the ABL gene occur usually at 5' of ABL exon a2, whereas those in the BCR gene are clustered in three regions. In most patients with CML and in approximately one-third of patients with Ph-positive ALL, the break occurs within the major breakpoint cluster region (M-bcr), spanning BCR exons e12 to e16 (originally referred to as exons b1 to b5), either between exons e13 and e14 or e14 and e15. These breakpoints produce BCR-ABL fusion genes that transcribe chimeric messenger RNA (mRNA) with either b2a2 or b3a2 junction encoding a p210BCR-ABL. In the remaining patients with Ph-positive ALL and in rare cases of CML (2), the breakpoint falls upstream of the M-bcr region, within the first intron of the BCR gene, known as the minor breakpoint cluster region (m-bcr). In these cases, BCR exon e1 is fused to ABL exon a2 and a chimeric mRNA with ela2 junction encoding a smaller p190BCR-ABL is formed. A third breakpoint location in the BCR gene has recently been identified downstream from the M-bcr region, defined as the micro breakpoint cluster region (μ-bcr) (2, 3). The resulting ela9a2 chimeric mRNA is translated into a larger p230BCR-ABL. It has been reported to be associated with a clinical picture of neutrophilic-CML characterized by a mild form of myeloproliferative disorder (3). CML expressing p190BCR-ABL is relatively rare, and to our knowledge only 17 cases were previously reported in the English literature (4-16).

In this article we describe a rare CML patient who showed prominent monocytosis with a low neutrophil/monocyte ratio in the peripheral blood, ela2 BCR-ABL transcript and lung cancer.

For editorial comment, see p 1092.

Case Report

A 77-year-old man was referred to the hospital in May 1997 for evaluation of leukocytosis. When the patient had been admitted to the hospital for the treatment of diabetes mellitus in February 1989, his white blood cell (WBC) count was 4.8x10^9/l with a normal differential count. On admission, the patient was afebrile and he had a palpable liver but no splenomegaly. Hematological findings were hemoglobin (Hb) 15.3 g/dl, platelets 353x10^9/l and WBC 33.8x10^9/l with 5% myelocytes, 2% metamyelocytes, 3% band-form neutrophils, 49% segmented neutrophils, 2% eosinophils, 3% basophils, 22% monocytes.

From the Department of Transfusion Medicine, Juntendo University School of Medicine, Tokyo, *the Department of Internal Medicine, Hitachi General Hospital, Ibaraki and **the Genomics Research Institute, SRL, Inc., Tokyo

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Reprint requests should be addressed to Dr. Akimichi Ohsaka, the Department of Transfusion Medicine, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421
and 14% lymphocytes. Cytochemical study showed that circulating monocytes were positive for \(\alpha\)-naphthyl butyrate esterase activity. The serum concentrations of C-reactive protein and bacterial cultures were all negative. The neutrophil alkaline phosphatase score was decreased to 111 (normal range in our hospital is 170–285). A bone marrow aspirate showed marked hypercellularity, myeloid hyperplasia (myeloid/erythroid ratio was 10.7) and megakaryocytic hyperplasia without an increase in blasts. Cytogenetic analysis of bone marrow cells by conventional G-banding showed 45,X,-Y,t(9;22)(q34;q11) in 19 of 20 metaphase cells and the remaining one cell was normal (data not shown). He was diagnosed with CML in the chronic phase and was treated with hydroxyurea, resulting in normal leukocyte counts but persistent monocytosis (20–30%).

In March 2000, the patient was admitted to the orthopedic department of the hospital because of lumbago and was diagnosed with lumbar spondylosis. Hematological findings were Hb 12.8 g/dl, platelets 108x10^9//l and WBC 6.0x10^9//l with 2% band-form neutrophils, 45% segmented neutrophils, 8% eosinophils, 22% monocytes and 23% lymphocytes. Subsequent physical examination showed a reddish skin nodule of 3 cm in diameter in his right chest wall, which was diagnosed pathologically as skin metastasis of adenocarcinoma. Computed tomography of the thorax revealed multiple nodular lesions in both lungs and bony destruction of ribs, suggesting a diagnosis of lung cancer. He died of disseminated intravascular coagulation and respiratory failure in April 2000. There was no evidence suggestive of blast crisis (BC) of CML in the course of the disease.

**Molecular Studies**

Reverse transcription-polymerase chain reaction (RT-PCR)

A highly sensitive nested PCR procedure was performed as described previously with some modification (13). RT-PCR analysis of bone marrow cells at diagnosis using \(BCR\) exon e13 and \(ABL\) exon a3 primers showed no amplification fragment (Fig. 1A). On the other hand, RT-PCR analysis using \(BCR\) exon e1 and \(ABL\) exon a3 primers revealed a 472-bp band (Fig. 1B), suggesting that the breakpoint in the \(BCR\) gene fell within the m-bcr region.

![RT-PCR analysis for BCR-ABL chimeric mRNA](image)

**Figure 1.** A. RT-PCR analysis for \(BCR\)-\(ABL\) chimeric mRNA (A) using \(BCR\) exon e13 and \(ABL\) exon a3 primers, and \(\beta\)-actin (B) as the internal control. Lane 1, present case; Lane 2, HL-60 leukemic cell line as the negative control; Lane 3, K562 leukemic cell line as the positive control for b3a2 fusion transcript; and M, DNA size marker. No specific band was amplified in Lane 1 of the patient. B. RT-PCR analysis for \(BCR\)-\(ABL\) chimeric mRNA (A) using \(BCR\) exon e1 and \(ABL\) exon a3 primers, and \(\beta\)-actin (B) as the internal control. Lane 1, present case; Lane 2, HL-60 leukemic cell line as the negative control; Lane 3, another patient with Ph-positive ALL carrying e1a2 fusion transcript; and M, DNA size marker. A 472-bp band was amplified in Lane 1 of the patient.
CML Expressing p190BCR-ABL

Sequencing analysis
The RT-PCR product was purified from agarose gel and sequenced using a Taq Dye Deoxy terminator cycle sequencing system (Perkin-Elmer, Foster City, CA). Sequencing analysis revealed that the 472-bp PCR product was derived from the fusion between BCR exon e1 and ABL exon a2 (e1a2, Fig. 2).

Discussion
We have described a rare CML patient carrying e1a2 BCR-ABL transcript who showed prominent monocytosis with a low neutrophil/monocyte ratio in the peripheral blood. The patient was associated with lung cancer during the course of the disease.

When high WBC counts are observed, monocytosis of higher than 1 x 10^9/l, which is a simple defining feature of chronic myelomonocytic leukemia (CMML) (17), is common in patients with chronic myeloproliferative disorder. To avoid misclassification of CML as CMML, it has been proposed that when leukocytosis is higher than 20 x 10^9/l, the absence of 'relat'ive monocytosis (<8%) should be a criteria for the diagnosis of CML (18). However, when leukocytosis is below 20 x 10^9/l, the absence of 'absolute' monocytosis (<1 x 10^9/l) is necessary for the diagnosis of CML (18). In the present study, we defined a monocytosis as an increase in both absolute number (>1 x 10^9/l) and percentage (>8%) of circulating monocytes, because

Table 1. Hematological Data and Clinical Characteristics in CML Patients Expressing p190BCR-ABL with Monocytosis*

<table>
<thead>
<tr>
<th>No.</th>
<th>Author (year)</th>
<th>ref. no.</th>
<th>Age/Sex</th>
<th>WBC (x10^9/L)</th>
<th>Monocytes (%)</th>
<th>basophils (%)</th>
<th>Immature granulocytes (%)</th>
<th>Neutrophil/Monocyte</th>
<th>Platelets (x10^9/L)</th>
<th>NAP score</th>
<th>Phase of diagnosis</th>
<th>Splenomegaly</th>
<th>Additional karyotype</th>
<th>Survival (months)</th>
<th>change</th>
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<td>Selleri (1990)</td>
<td>(4)</td>
<td>44/F</td>
<td>56.8</td>
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<td>2</td>
<td>19</td>
<td>5.3</td>
<td>510</td>
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<td></td>
</tr>
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<td>4</td>
<td>27</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>6.5</td>
<td>0</td>
<td>16</td>
<td>3.0</td>
<td>335</td>
<td>Low CP</td>
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<td>No</td>
<td>21, Died</td>
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<td></td>
</tr>
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<td>Yamaguchi (1998)</td>
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<td>7.5</td>
<td>1.5</td>
<td>14</td>
<td>3.8</td>
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<td>NA BC(1)</td>
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<td></td>
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<td>25</td>
<td>1.2</td>
<td>88</td>
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<td></td>
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<td>3</td>
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<td>4.6</td>
<td>298</td>
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<td>70/M</td>
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<td>7.0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>305</td>
<td>Low BC(1)</td>
<td>+</td>
<td>No</td>
<td>25, Died</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Ravandi (1999)</td>
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<td>49/F</td>
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<td>22.8</td>
<td>13</td>
<td>NA</td>
<td>4.8</td>
<td>33</td>
<td>Low CP</td>
<td>+</td>
<td>No</td>
<td>25, Died</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>8</td>
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<td>5.0</td>
<td>1124</td>
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<td>+</td>
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<td>0</td>
<td>7</td>
<td>2.4</td>
<td>353</td>
<td>Low CP</td>
<td>-</td>
<td>Y</td>
<td>35, Died</td>
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<td></td>
</tr>
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</table>

*Monocytosis was defined as an increase in both absolute number (>1 x 10^9/l) and percentage (>8%) of peripheral blood monocytes in this study. Biphonotypic blast crisis. WBC: white blood cells, NA: not available, NAP: neutrophil alkaline phosphatase, CP: chronic phase, AP: accelerated phase, BC: blast crisis.

Table 2. Hematological Data and Clinical Characteristics in CML Patients Expressing p190BCR-ABL without Monocytosis*

<table>
<thead>
<tr>
<th>No.</th>
<th>Author (year)</th>
<th>ref. no.</th>
<th>Age/Sex</th>
<th>WBC (x10^9/L)</th>
<th>Monocytes (%)</th>
<th>basophils (%)</th>
<th>Immature granulocytes (%)</th>
<th>Neutrophil/Monocyte</th>
<th>Platelets (x10^9/L)</th>
<th>NAP score</th>
<th>Phase of diagnosis</th>
<th>Splenomegaly</th>
<th>Additional karyotype</th>
<th>Survival (months)</th>
<th>change</th>
</tr>
</thead>
<tbody>
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<td>77/F</td>
<td>63.3</td>
<td>4</td>
<td>2.5</td>
<td>1</td>
<td>12.5</td>
<td>261</td>
<td>Low CP</td>
<td>- +</td>
<td>No</td>
<td>27, Died</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Guo (1993)</td>
<td>(7)</td>
<td>52/M</td>
<td>180</td>
<td>10.8</td>
<td>13</td>
<td>24</td>
<td>8.3</td>
<td>187</td>
<td>NA CP</td>
<td>+</td>
<td>No</td>
<td>20, Alive</td>
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<td></td>
</tr>
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<td>Kunieda (1994)</td>
<td>(9)</td>
<td>62/M</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>843</td>
<td>Low CP</td>
<td>+</td>
<td>No</td>
<td>36, Died</td>
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<td>0.3</td>
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<td>1</td>
<td>2.5</td>
<td>77</td>
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<td>5</td>
<td>Kirk (1996)</td>
<td>(11)</td>
<td>47/M</td>
<td>225</td>
<td>2</td>
<td>4.5</td>
<td>0</td>
<td>15</td>
<td>37.5</td>
<td>NA CP</td>
<td>+</td>
<td>No</td>
<td>Died</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Solves (1999)</td>
<td>(14)</td>
<td>31/F</td>
<td>268</td>
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<td>1</td>
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<td>Low CP</td>
<td>+</td>
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<tr>
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<td>35</td>
<td>542</td>
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<td>+</td>
<td>inv(3)(q22q26), -17, -22, others</td>
<td>3, Died</td>
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</table>

*Monocytosis was defined as an increase in both absolute number (>1 x 10^9/l) and percentage (>8%) of peripheral blood monocytes in this study. Biphonotypic blast crisis. WBC: white blood cells, NA: not available, NAP: neutrophil alkaline phosphatase, CP: chronic phase, AP: accelerated phase, BC: blast crisis.

Internal Medicine Vol. 41, No. 12 (December 2002) 1185
monocytosis is dependent on total WBC counts.

CML with e1a2 BCR-ABL transcript is relatively rare, and to our knowledge it has been reported previously in only 17 cases whose clinical data were available (4–16). Ten of the 18 reported cases (including the present case) showed prominent monocytosis with a low neutrophil/monocyte ratio in the peripheral blood (Table 1), and the remaining 8 cases had no monocytosis (Table 2). The hematological and clinical parameters in the CML patients with monocytosis (n=10, group A) were compared with those in the CML patients without monocytosis (n=8, group B). Although the progression to BC in the group A patients was not different from that in the group B patients, the type of BC was characteristic. Three of the 4 group A patients with BC showed biphenotype, whereas 2 of the 3 group B patients with BC exhibited lymphoid phenotype. Therefore, the involvement of lymphoid lineage was observed in 5 of the 7 patients with BC. In addition, 2 of the 8 group B patients showed additional chromosomal abnormalities except for the loss of the sex chromosomes. No differences between the 2 groups were observed in relation to other hematological and clinical characteristics. Melo et al (8) have suggested that p190BCR-ABL CML may be a specific form of CML, in which the cytomorphological characteristics are intermediate between CML and CMML. However, 8 of the 18 reported cases showed the characteristic features more commonly found in the classical CML without prominent monocytosis, indicating that CML expressing p190BCR-ABL is a heterogeneous disease. It is premature to claim any conclusion regarding the relationship between genotype and leukemia phenotype in the p190BCR-ABL CML. Further studies of more cases are needed to clarify the issue.

The p190BCR-ABL transcript is more frequently associated with Ph-positive ALL than CML. The higher transforming ability of p190BCR-ABL (19) can cause an immediate transformation of the lymphoid progenitor cells originating from the abnormal stem cells. However, it has been shown that p190BCR-ABL transcript is frequently expressed at low levels using competitive PCR assays in p210BCR-ABL CML, although its pathogenetic significance is uncertain (20). In addition, there are sporadic cases of chronic phase CML expressing only p190BCR-ABL (4–16). Although we have no clear explanation why these cases do not progress rapidly towards ALL, the possibility is that a molecular background, genetically determined, is able to block, at least partially, the transforming ability of p190BCR-ABL (21). Alternatively, three forms of BCR-ABL transcript (p190BCR-

\[ \text{ABL}, \text{p210BCR-ABL, and p230BCR-ABL} \] might have identical leukemogenic properties, but their expression might be largely restricted to different hematopoietic lineages because a particular breakpoint in the BCR gene is favored in a given lineage during the formation of the Ph. The BCR intron 1 breakpoint might be frequent in B lymphoid progenitor cells but uncommon in hematopoietic stem cells, explaining the rarity of p190BCR-ABL in CML (22). Therefore, p190BCR-ABL per se can induce the myeloproliferative process including granulocytic and monocytic lineages, resulting in promotion of chronic phase CML. This is supported by experiments which used a murine bone marrow transduction/transplantation model, where the three oncogenes induced an identical fatal CML-like disease in recipient mice, with no appreciable differences in histopathology, hematologic parameters or disease latency (22).

Another issue of this study is that the present case was associated with lung cancer during the course of the disease. Nagura et al (23) have reported multiple primary cancers associated with hematological malignancies in Japanese patients. There was no association between CML and lung cancer in the 231 cases collected (23). This suggests that the association of the two diseases found in our patient may be coincidental.

References

CML Expressing p190\textsuperscript{BCR-ABL}


