Short communication

A case of chronic myelogenous leukemia with e8a2 fusion transcript

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Abstract

The Philadelphia chromosome and its corresponding fusion gene, \textit{BCR-ABL}, is one of the best-known genetic abnormalities in hematological malignancies. Major \textit{BCR-ABL} translocation is much more common in chronic myelogenous leukemia (CML) and minor \textit{BCR-ABL} in acute lymphoblastic leukemia. We experienced an extraordinarily rare case of CML with an e8a2 variant. An unusual band, other than the common transcripts, was observed in reverse transcription polymerase chain reaction (RT-PCR) for the \textit{BCR-ABL} gene rearrangement. Sequence analysis of the PCR product revealed an 1172-bp e8a2 fusion with a 14-bp insertion of \textit{ABL} intron 1a. The patient achieved a complete hematological response 3 months after imatinib treatment. It is necessary to keep in mind that an unexpected band revealed with RT-PCR may mean the presence of unusual fusion gene.

1. Introduction

Philadelphia chromosome (Ph), characterized by the t(9;22)(q34;q11.2) translocation, is observed in $>95\%$ of chronic myelogenous leukemia (CML) cases\cite{1,2}. In general, the \textit{BCR-ABL} fusion gene is transcribed as a large chimeric RNA, which typically results in either or both of b2a2 (e13a2) and b3a2 (e14a2) fusion mRNAs, both or which are translated into p210 BCR-ABL protein. The protein plays a crucial role in the pathogenesis of CML in the chronic phase\cite{3}. A minor proportion of CML patients express unusual types of \textit{BCR-ABL} transcripts, although most CML patients have b2a2 or b3a2. Recently, several patients with unusual e8a2 \textit{BCR-ABL} transcripts have been reported and characterized\cite{4--7}. Here, we report a case of CML with a novel e8a2 \textit{BCR-ABL} transcript with a 14-bp fragment insertion of the \textit{ABL} intron 1a.

2. Materials and methods

2.1. Case report

A 46-year-old man, who had been referred from a secondary-care hospital because of high white blood cell (WBC) count with a left shift and palpable spleen, was admitted to Ajou University Hospital and diagnosed with a chronic phase of CML. On admission, his complete blood count revealed white blood cells 46,200/\text{mL} (normal, 3,900--9,690) with 4\% of blast cells, hemoglobin 13.0 g/\text{dL} (normal, 11.7--17.1), and platelets 663,000/\text{mL} (normal, 134,000--387,000). Peripheral blood and marrow findings were compatible with CML. Conventional cytogenetic analysis was not performed, because of a missing order. Instead, dual-color, dual-fusion fluorescence in situ hybridization (FISH) on peripheral blood was done to test for the presence of \textit{BCR-ABL} rearrangement or t(9;22). Multiplex reverse transcription--polymerase chain reaction (RT-PCR) for \textit{BCR-ABL} gene rearrangement showed an unexpected band, in addition to the typical fusion transcripts (Fig. 1A), whereas FISH analysis showed a typical translocation pattern consistent with CML. Sequence analysis of the PCR product revealed an 1172-bp e8a2 fusion with a 14-bp insertion of \textit{ABL} intron 1a (Fig. 1B). We started administration of imatinib mesylate at a daily dose of 400 mg, and a complete hematological response was achieved by 3 months. The patient has been maintained well on imatinib at a daily dose of 300 mg.

2.2. FISH analysis

Interphase FISH analysis using Vysis dual-color, dual-fusion, locus-specific identifiers (LSI) BCR and ABL probes (Abbott Molecular/Vysis, Des Plaines, IL) was
performed according to the manufacturer’s recommendations. The slides were freshly prepared from the patient’s peripheral blood, and 200 interphase nuclei were counted.

2.3. Molecular studies

2.3.1. Multiplex RT-PCR

The multiplex RT-PCR assay was performed according to the manufacturer’s instructions with a Seeplex leukemia kit (Seegene, Seoul, Korea), which is designed to detect eight common BCR–ABL transcripts, including the major breakpoint cluster region (M-bcr), minor bcr (m-bcr), and micro bcr (µ-bcr). Cycling conditions were as follows: 94°C for 15 minutes (1 cycle); 94°C for 30 seconds, 60°C for 1 minute 30 seconds, 72°C for 1 minute 30 seconds (37 cycles); and 72°C for 10 minutes (1 cycle). The PCR products were analyzed by 2% agarose gel electrophoresis at 100 V for 30 minutes.

2.3.2. Cloning and sequencing

The purified PCR product (~1200 bp) was inserted into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA) according to the manufacturer’s specifications. Plasmid DNA was prepared using a Qiagen plasmid mini-kit (Hilden, Germany). Sequencing was performed using an ABI BigDye terminator version 3.1 cycle sequencing kit (PE Applied Biosystems, Foster City, CA) and the M13F/M13R primer on an ABI 310 DNA sequencer. Sequence analysis was done using the basic local alignment search tool BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and Ensembl (http://www.ensembl.org/index.html).

3. Results

Interphase FISH showed a typical translocation pattern (i.e., 2 yellow BCR–ABL fusion signals, 1 green BCR signal, and 1 orange ABL signal) in 65% of nuclei counted. Multiplex RT-PCR for BCR–ABL showed an unusually large band (~1200 bp), in addition to the internal control band (Fig. 1A). Sequencing revealed that this band corresponded to a junction between BCR exon e8 and ABL exon a2 including a 14-nucleotide fragment insertion from ABL intron 1a at the fusion point (nt 88286–88299, GenBank NW_924573 [http://www.ncbi.nlm.nih.gov]) (Fig. 1B).

4. Discussion

Several unusual BCR–ABL transcripts are uncommon in both CML and acute lymphoblastic leukemia, including e2a2 [8], e3a2 [9], e6a2 [3], e8a2 [4,6,7], e15a2 [10], e19a2 [11,12], e1a3 [13], e13a3 [14], and e14a3 [15]. Including the present case, 13 CML cases with e8a2 junction more frequent than other variants have been reported to date. The e8a2 is an example of transcripts with fusion of out-of-frame ABL and BCR exons [16]. The direct junction between BCR exon e8 and ABL exon a2 would not produce an oncogenic BCR–ABL protein due to a generation of a premature stop codon (UAG) at position 7 after the fusion [5]. Therefore, either the chromosomal breakpoint must occur inside exons or intronic sequences must be interposed to restore the reading frame. In previously reported cases, e8a2 mRNA revealed various sequences by an insertion of intronic sequences generally derived from the ABL intron Ib, by breakpoints within the BCR exon 8 or the ABL exon a2, or by both mechanisms [4–7].

The most frequent rearrangement in patients with e8a2 revealed by molecular study is an identical 55-bp inverted fragment insertion from ABL intron Ib; this accounts for >50% of the e8a2 patients described to date. Demehri et al. [5] reported a case of CML with e8a2 having an 151-bp insertion of ABL intron 1a and breakpoint within the exon a2. The present case showed a breakpoint within e8 exon and a 14-bp fragment insertion from ABL intron 1a. The present case thus appears to be the second case with e8a2 transcript showing an ABL intron 1a insertion.

For the present it seems that e8a2-positive CML patients have no distinctive clinical features except for good response to imatinib. Because the number of cases hitherto...
reported is small, collecting more cases is necessary to characterize e8a2-positive CML more definitively. Patients with e8a2 transcripts were reported to be resistant to interferon-α treatment, suggesting a worse prognosis of CML with e8a2 fusion gene [5]. After the introduction of imatinib mesylate, imatinib-resistant e8a2 transcripts were reported to be resistant to interferon treatment, suggesting a worse prognosis of CML with e8a2 CML might be sensitive to the ABL tyrosine kinase inhibitor, imatinib [7,17]. The present case showed a good response to imatinib, too.

A variety of multiplex RT-PCR assays are now available for BCR–ABL fusion transcripts, to detect not only the common BCR–ABL fusion (M-bcr, m-bcr) but also unusual variants. In clinical laboratories using optimal reagents and procedures, an aberrant band in multiplex RT-PCR for BCR–ABL may indicate the presence of unusual fusion gene, and the identification of the atypical transcript by DNA sequencing may be necessary to further validate the finding.

References