Cancer Genetics 209 (2016) 161–165

Cancer Genetics

SHORT COMMUNICATION

Adult acute lymphoblastic leukemia with a rare b3a3 type BCR/ABL1 fusion transcript

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The Philadelphia chromosome (Ph) is the most frequent chromosomal abnormality detected in adult acute lymphoblastic leukemia (ALL). This chromosome forms the *BCR/ABL1* fusion gene; thus, *ABL1* exon a2 is generally used as a primer-binding region for the detection of the fusion transcript via reverse transcription polymerase chain reaction (RT-PCR). We observed a rare case of adult Ph-positive (Ph$^+$) ALL, in which the BCR/ABL1 fusion transcript was not detected using the *ABL1* exon a2 region primer. However, we were able to isolate a PCR product by RT-PCR with the *BCR* exon 13 (b2) and *ABL1* exon a3 primers. Analysis of the sequence of the RT-PCR product revealed that the fusion point was between *BCR* exon 14 (b3) and *ABL1* exon a3, and that the transcript lacked *ABL1* exon a2. The patient achieved cytogenetic remission through combination chemotherapies, but relapse occurred before hematopoietic stem cell transplantation and the patient died 11 months after the initialization of chemotherapies. If the BCR/ABL1 fusion transcript is undetected with the *ABL1* exon a2 region primer in Ph$^+$ ALL cases, an RT-PCR analysis that can detect the b3a3 type BCR/ABL1 fusion transcript should be considered to improve diagnosis.

Keywords Acute lymphoblastic leukemia, Philadelphia chromosome, BCR/ABL1 fusion transcripts

The Philadelphia chromosome (Ph) is the most frequent chromosomal abnormality detected in adult acute lymphoblastic leukemia (ALL), and Ph-positive (Ph$^+$) ALL has been associated with unfavorable outcome in both adults and children (1). The Ph chromosome results from a reciprocal translocation of chromosomes 9 and 22 [t(9;22)(q34;q11.2)]. This translocation generates a fusion of the *BCR* and *ABL1* genes and results in the constitutive activation of the *ABL1*-encoded tyrosine kinase. In ALL, the frequency of BCR/ABL1 fusion transcripts increases with age, a characteristic that was substantiated with the finding that 52.7% of a cohort of 50–60 year-old ALL cases were discovered to have Ph chromosomes (2).

There are diverse breakpoints that generate BCR/ABL1 fusion transcripts. However, three main cluster regions of the *BCR* gene, referred to as the major, minor, and micro (or M-, m-, and μ-, respectively) regions, have been reported (3). The German Multicenter ALL (GMALL) study reported that in ALL cases expressing BCR/ABL1 fusion transcripts the detection frequencies for the M-*BCR*, m-*BCR*, and those having both M- and m-*BCR* breakpoint regions were 31.2%, 66.2%, and 0.17%, respectively. However, no μ-*BCR* cases were reported (4). Because the majority of these *BCR* breakpoint regions bind to the upstream regions of *ABL1* exon a2 (3), *ABL1* exon a2 is generally selected as the primer binding region for the detection of the *BCR/ABL1* fusion transcript by reverse transcription polymerase chain reaction (RT-PCR), especially for detecting major-*BCR/ABL1* fusions (5,6). Therefore, *BCR/ABL1* fusions lacking the *ABL1* exon a2 region may have gone undetected by conventional RT-PCR methods in some Ph$^+$ ALL cases.

We discovered a case of adult Ph$^+$ ALL that exhibited a fusion of *BCR* exon 14 (b3) and *ABL1* exon a3, which lacked *ABL1* exon a2. To our knowledge, there are only three reported cases of this rare fusion transcript: two in children (7,8)

Received July 22, 2015; received in revised form November 2, 2015; accepted December 31, 2015.

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http://dx.doi.org/10.1016/j.cancergen.2015.12.014

and one in adult (9). Thus, the present case is only the second report of this abnormality in adult Ph^+ ALL.

Case study

A 43-year-old man was admitted to the clinic due to general fatigue in December 2008. Blood tests revealed a marked increase in white blood cells, and the patient was admitted to our institution in January 2009. There were no remarkable findings noted in the patient's or his family's medical histories, and a physical examination indicated that neither the spleen nor the lymph nodes were palpable. Peripheral blood testing established a white blood cell count of 203.0×10^9/L (band neutrophils 0.5%, segmented neutrophils 0.5%, lymphocytes 9.5%, monocytes 0.5%, and blast cells 89.0%), hemoglobin levels of 9.3 g/dL, a platelet count of 66×10^9/L, and a lactate dehydrogenase level of 540 IU/L.

A bone marrow examination revealed hypercellularity with 97.4% myeloperoxidase-negative blast cells. The immunophenotypes of these blast cells from flow cytometry were CD10-, CD19-, and CD20-positive and CD3-, CD13-, and CD33-negative. Furthermore, a chromosomal analysis of the bone marrow confirmed a Philadelphia translocation t(9;22)(q34;q11.2). Based on these results, the patient was diagnosed with Ph^+ acute lymphoblastic leukemia (Ph^+ ALL).

In accordance with the Ph-positive ALL 208 protocol of the Japan Adult Leukemia Study Group (JALSG) (10), the patient received chemotherapy consisting of cyclophosphamide, daunorubicin, vincristine, prednisolone, and imatinib mesylate. The patient achieved cytogenetic remission and was negative for the *BCR/ABL1* fusion signal by fluorescence in situ hybridization (FISH). Molecular remission was not evaluated. As the patient was considered a high-risk ALL case, he was transferred to another institution to receive allogeneic hematopoietic stem cell transplantation (HSCT). However, early relapse occurred before HSCT and several therapies including dasatinib, a second-generation tyrosine kinase inhibitor, were attempted but failed to achieve a second remission. HSCT was performed without remission in August 2009 and remission was temporarily achieved, but the ALL rapidly progressed and the patient died in November 2009. Written informed consent was obtained from the family for the publication of this case report with accompanying images.

Materials and methods

Cytogenetic analysis and fluorescence in situ hybridization (FISH)

Bone marrow cells were cultured and twenty chromosomes in metaphase were analyzed using the standard laboratory G-banding protocol from SRL, Inc. (Tokyo, Japan). Signals from FISH of *BCR/ABL1* fusions were analyzed by Vysis LSI BCR/ABL1 dual color, dual fusion translocation probe set (Abbott Molecular Inc., Des Plaines, IL) according to the manufacturer's recommended protocol (SRL, Inc.). These probes hybridized to chromosome 22q11.2 (*BCR*-spectrum-green) and chromosome 9q34 (*ABL1*-spectrum-red) and more than 100 cells in interphase were analyzed.

RNA isolation and real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR)

Real time RT-PCR was performed by Biomedical Laboratories, Inc. (Kawagoe, Japan) according to the methods of Osumi et al. (11). Briefly, total RNA was extracted from bone marrow cells using the QIAamp RNA blood mini kit (Qiagen GmbH, Hilden, Germany) and transcribed into cDNA in a reaction mixture comprising random hexamers (Gibco-BRL, Rockville, MD), reverse transcriptase (SuperScript II; Gibco-BRL), RNase inhibitor (Gibco-BRL), and dNTPs.

To detect the major, minor, and micro *BCR/ABL1* (M-, m-, and μ-*BCR/ABL1*, respectively) fusions, the *BCR* primer regions were exon 13 (b2) (5′-GATGCTGACCAACTCGTGTGTG-3′) for M-*BCR/ABL1*, exon 1 (5′-ATCGTGGGCGTCCGCAAGAC-3′) for m-*BCR/ABL1*, and exon 19 (c3) (5′-ATGGAGGAG GTGGGCATCTAC-3′) for μ-*BCR/ABL1*. The *ABL1* primer regions were exon a2 (5′-TGGCCACAAAATCATACAGTGC-3′) for M-*BCR/ABL1*, exon a2 (5′-GCTCAAAGTCAGAT GCTACTG-3′) and a3 (5′-GGCTTCACACCATTCCCCAT-3′) for m-*BCR/ABL1*, and exon a2 (5′-GCTCAAAGTCAG ATGCTACTG-3′) for μ-*BCR/ABL1*.

All of the TaqMan probes designed to bind *ABL1* exon a2 and the sequences for M-, m-, and μ-*BCR/ABL1* were (5′-CCTTCAGCGGCCAGTAGCATCTGACTTT-3′), (5′-CGC CCTCGTCATCGTTGGGCCAGATCT-3′), and (5′-ACATCC AGGCACTGAAGGCAGCCTTCGA-3′), respectively. Target DNA amplification was performed with these primers and probes in 1× TaqMan Universal PCR Master Mix (Applied Biosystems [ABI], Foster City, CA). The reaction products were analyzed using a PRISM7900 system with Sequence Detection System software (ver. 1.6.3, ABI).

Direct sequencing

For direct sequencing analysis, RNA isolation and cDNA transcription were carried out with bone marrow cells by the same aforementioned methods. The *BCR* exon13 (b2) region-targeting forward primer (5′-GATGCTGACCAACTCGTGTGTG-3′) and *ABL1* exon a3 region-targeting reverse primer (5′-GGCTTCACACCATTCCCCAT-3′) were used for PCR amplification. The PCR products were analyzed by capillary electrophoresis and purified by AMPure (Beckman Coulter, Brea, CA). Direct DNA sequencing employing the same primers used for PCR was performed with the Big Dye Terminator v.3.1 cycle sequence kit (ABI) and an ABI 3130 Genetic Analyzer (ABI). DNASIS software (Hitachi Solutions, Tokyo, Japan) was used for analysis. All of our primers, including primers described in the real time RT-PCR section, were originally designed by Biomedical Laboratories, Inc. (Kawagoe, Japan) using Primer-BLAST and were different from those used by BIOMED-1 (12). The primers in this study have been used for clinical diagnosis in Japan since 2002, although a precise comparative study with BIOMED-1 primers has not been conducted.

Results

G-banding analysis from the first diagnosis revealed that 17 out of 20 cells exhibited 46,XY,del(9)(p?) and t(9;22)(q34;q11.2) (Figure 1A). FISH analysis of peripheral blood cells showed