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Research paper

A rare e13a3 (b2a3) BCR-ABL1 fusion transcript with normal karyotype in chronic myeloid leukemia: The challenges in diagnosis and monitoring minimal residual disease (MRD)

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ABSTRACT

Patients with chronic myeloid leukemia (CML) have a t (9;22)(q34;q11.2) or variant translocation that results in a BCR-ABL1 fusion gene. For many years, conventional karyotyping has been used as the standard diagnostic tool for t (9;22) (q34;q11.2). However, it has several limitations that may lead to failure for detecting BCR-ABL1 gene rearrangements in around 5% of all CML patients. Although reverse transcription polymerase chain reaction (RT-PCR) has evolved as a sensitive method for detecting BCR-ABL1 translocation, this method fail to detect certain BCR-ABL1 fusion transcript type, such as e13a3 (also known as b2a3), as a result of many commercially available and laboratory-developed primer sets. Fortunately, these two rare situations rarely appear at the same time, therefore, the combination of two methods rarely misdiagnosed the patients with CML. In this study, we report a patient with CML who tested both negative by RT-PCR and cytogenetic analysis at the time of diagnosis. She was diagnosed as atypical CML (aCML) and allogeneic hematopoietic stem cell transplantation was suggested. Further fluorescence in situ hybridization (FISH) showed cryptic insertion of ABL into BCR gene on chromosome 22, and DNA sequencing with alternative primer sets demonstrated the presence of an e13a3 BCR-ABL1 fusion. She was diagnosed as CML and received imatinib 400 mg/day. A follow-up BCR-ABL1 FISH analysis demonstrated a markedly reduced BCR-ABL1 fusion rate of 0 after 6 months treatment, indicating a complete cytogenetic response.

1. Introduction

More than 90% of all CML and around 10–25% of acute lymphoblastic leukemia (ALL) cases are associated with a translocation between chromosome 9 and 22 [1,2]. This translocation generates 2 novel fusion genes: the breakpoint cluster region/receptor tyrosine kinase gene BCR/ABL on the derivative chromosome 22q, known as the Ph chromosome, and a reciprocal ABL/BCR fusion gene on the derivative chromosome 9q (der 9). In CML, over 95% of the breakpoints involve the major breakpoint cluster region (M-bcr) of BCR introns downstream of either exon 13 or 14 and introns downstream of ABL exon 2, resulting in fusion transcripts e13a2 (b2a2) and e14a2 (b3a2), which produces a 210 KD BCR/ABL protein that contributes to CML pathogenesis. The minor breakpoint cluster region (m-bcr) in the BCR gene is a less common (but typically seen in acute lymphoblastic leukemia) breakpoint involving the exon 1, fused to ABL a2, resulting in a e1a2 fusion transcript, which encodes a 190 KD BCR/ABL fusion protein. BCR exons 19 and 20, known as μ-bcr, fuses to ABL a2, encoding a 230 KD fusion protein (e19a2) [3–5]. Rare atypical breakpoints have also been sporadically reported, such as BCR breakpoints occurring within exons rarely fused to ABL a3, which were apt to misdiagnose [5].

Detection of t (9;22) (q34;q11.2) translocation is important in patients with CML and karyotyping technique is one of the robust assays prescribed even today for newly diagnosed CML patients. The method has advantage of very high specificity and can detect additional chromosomal aberrations also since the entire set of chromosomes comes under the analysis purview. However, the Ph chromosome is not demonstrable by cytogenetic studies in about 5% to 10% of CML patients and these patients are classified as having Ph-negative CML, such as cryptic insertion of ABL into BCR gene on chromosome 22 [6–8].

With time, RT-PCR has evolved as a sensitive method for detecting BCR/ABL translocation. However, this method fail to detect certain

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BCR-ABL1 fusion transcript type, such as e13a3, as a result of many commercially available and laboratory-developed primer sets do not cover such rare fusions [5]. To our knowledge, there are no reports of CML patients presented both with normal karyotype and RT-PCR at the same time so far, where Masuko et al. published a similar case with the only exception that the karyotype was abnormal but atypical (Ph chromosome negative) [9]. Therefore, RT-PCR can be used successfully in combination with cytogenetic studies to diagnose most of Ph-negative CML.

Unfortunately, if patient with CML is tested negative both by RT-PCR and cytogenetic analysis at the same time, further methods for detecting those rare translocations are needed. FISH analysis using dual color BCR/ABL translocation probes allows the visualization of BCR/ABL rearrangement in both interphase and metaphase cells, and the presence of the BCR/ABL fusion gene on chromosome 22 has been reported in a substantial subset of these patients. Some studies demonstrated the efficiency of FISH in detecting BCR/ABL fusion in CML with masked or variant Ph chromosome, which is often not apparent with conventional karyotyping [8,10]. On the other hand, for patients harbored rare types of transcription, such as e13a3, FISH can effectively identify atypical BCR/ABL fusion signals to clarify a diagnosis and quantify minimal residual disease (MRD) for these patients.

Here we report a case of CML with an e13a3 fusion transcript and cryptic insertion of ABL into BCR gene on chromosome 22 that showed a positive BCR-ABL1 FISH result but had a Ph chromosome negative karyotype and negative result by RT-PCR. To our knowledge, this is the first report of such an extremely rare BCR/ABL fusion variant around the world so far.

2. Materials and methods

2.1. Metaphase cytogenetics and fluorescence in situ hybridization

Bone marrow cells from the patient were cultured in vitro according to standard laboratory protocol. Twenty G-banded metaphases were analyzed respectively. FISH was performed following the manufacturer's recommended protocol, which using BCR/ABL1 D-FISH probe set (GPmedical technologies, Ltd.). A normal cell should show two separate sets of red and green signals (2R2G), while a cell containing reciprocal t (9;22) (q34;q11.2) shows individual red and green signals from the normal 9 and 22 chromosomes and two red/green fusion signals from the derivative 9 and 22 chromosomes (1R1G2F pattern). Positive and negative controls plus 200 interphase cells from the patient were screened and analyzed.

2.2. RT-PCR for detecting BCR/ABL transcript

Total RNA was isolated from patient bone marrow cells. Isolated RNA was quantified by spectrophotometric analysis at 260 and 280 nm and transcribed into cDNA in a reaction mixture comprising random hexamers, reverse transcriptase, RNase inhibitor, and dNTPs.

Routine BCR/ABL transcript detection was performed using the BCR/ABL protocol. This kit allows amplification of BCR/ABL and ABL transcripts in two independent reactions. The M-BCR, m-BCR and μ–BCR regions are targeted and the breakpoints covered are e13a2 (b2a2) and e14a2 (b3a2) in the M-BCR while e1a2 in the m-BCR region and e19a2 in theμ–BCR.

2.3. Direct sequencing

RNA isolation and cDNA transcription were carried out with bone marrow cells by the same aforementioned methods. The BCR exon13 region-targeting forward primer (5'- CATCCGGGAGCAGCAGAAGAA −3') and ABL exon a3 region-targeting reverse primer (5'-GTGTTTCTCCAGACTGTTGGCT −3') were used for PCR amplification. PCR products were purified using Qiaquick reagents (Qiagen) and were cycle sequenced using Big Dye v3.1 reagents (Applied Biosystems). Automated sequencing performed by capillary electrophoresis on an ABI3500 (Applied Biosystems). Sequences were aligned and examined using lasergene SeqMan software ver. 7.1 (DNAStar, Inc.).

2.4. Case report

A 24 year-old female with elevated white blood cell count (WBC) for two months before referred to our hospital. Blood routine test showed an elevated WBC of 51,000/mm^3 and platelet count of 500,000/mm^3 without anemia. Her peripheral blood smear showed left-shifted but no present evidence of acute leukemia. She was referred to other hospital pursuing a work up for suspected myeloproliferative neoplasm, specifically directed toward a diagnosis of CML. She underwent three times of bone marrow aspirate and biopsy in two hematological centers, and both karyotyping and RT-PCR testing (including three most common BCR/ABL breakpoints and rare breakpoint of μ-BCR) were negative. She was diagnosed as aCML and allogeneic hematopoietic stem cell transplantation was suggested.

3. Results and discussion

After a complete history, physical, and review of the patient's outside records and report, the diagnosis remained consistent with aCML. A repeat bone marrow aspiration and biopsy with complete morphological, cytological and molecular testing was performed. Bone marrow biopsy test showed a markedly hyper-cellular marrow with myeloid predominance. Cytogenetic analysis presented a normal karyotype of 46, XX [20] (Fig. 1). RT-PCR was performed to target all common BCR/ABL breakpoints, including e13a2, e14a2, e1a2 and e19a2. Repeat RT-PCR tests for all these loci were found to be negative. FISH analysis identified BCR/ABL translocation with 72% of cells showing imbalanced translocation between chromosome 9 and 22 and with 2R1G1F pattern (Fig. 1), which meant cryptic insertion of ABL into BCR gene on chromosome 22. In view of the confirmed abnormal result detected in FISH coupled with normal karyotype and absence of common BCR/ABL breakpoints, we proceeded to test for the presence of rare variants of BCR/ABL gene fusion.

Thermal amplification of the e13a3-harboring BCR-ABL1 hybrid cDNA generated a 250 bp PCR amplicon. We sequenced the amplicon using both the forward and the reverse primers. Results indicated that the break occurred in nucleotide position 3303 of the BCR (GenBank Y00661) and 618 (GenBank M14752) of the ABL gene, making this case a rare e13a3 category of BCR/ABL translocation (Fig. 2). So she was diagnosed as CML and received imatinib 400 mg/day. A follow-up BCR-ABL1 FISH analysis demonstrated a markedly reduced BCR-ABL1 fusion rate of 0 after 6 months treatment, indicating a complete cytogenetic response (CCyR). So far, the patient has maintained CCyR for more than two years. Molecular response could not be assessed due to the persistently negative RT-PCR results.

According to the literature, two alternative mechanisms were postulated to elucidate the formation of a fusion gene in Ph-negative but BCR/ABL-positive CML patients. The first mechanism involves a one-step model where BCR/ABL results from a simple insertion of either ABL1 into BCR or BCR into ABL after three genomic breaks. The second mechanism is a multiple-step model involving an initial classical t (9;22)(q34;22) followed by a second translocation of the two products and/or a third chromosome, requiring a minimum of 4 genomic breaks [8,11]. In our case, FISH results led to the conclusion that cryptic insertion of ABL into BCR gene on chromosome 22.

In addition to being a rare Ph-negative CML case, our patient also harbors a rare e13a3 type of BCR/ABL translocation which routine RT-PCR test using commercial kits that do not cover such rare fusions will generate a false negative result. If FISH was not performed at the time of diagnosis, she would be misdiagnosed as aCML and treated by