Detection of a novel CBFB/MYH11 variant fusion transcript (K-type) showing partial insertion of exon 6 of CBFB gene using two commercially available multiplex RT-PCR kits

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Abstract

We report on a 20-year-old man with acute myeloid leukemia (AML) showing a distinct novel CBFB/MYH11 variant fusion transcript. Initial results of bone marrow, chromosome, and flow cytometric analyses were not in accordance with the diagnosis of acute myelomonocytic leukemia with eosinophilia (AML-M4Eo) or AML with a CBFB/MYH11 rearrangement. However, results from 2 commercially available multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) tests repeatedly showed an unusual PCR product from his bone marrow specimen. Not only does this case show a partial insertion of exon 6 of the CBFB (ENSG00000067955) gene, but it also involves novel breakpoints within both exon 6 of the CBFB gene and exon 28 (previously exon 7) of the MYH11 (ENSG00000133392) gene, which is regarded as a previously non-reported, new type (K-type) of CBFB/MYH11 fusion transcript. In addition, our study result was in agreement with the recent report of Schnittger et al. that rare fusion transcripts of CBFB/MYH11 are correlated with an atypical cytomorphology and other aberrant characteristics. Therefore, multiplex RT-PCR and sequence analysis of these atypical products should be performed to diagnose atypical AML with CBFB/MYH11 rearrangement, to predict prognosis of these patients as well as to elucidate the molecular mechanism. © 2009 Elsevier Inc. All rights reserved.

1. Introduction

Acute myeloid leukemia (AML) with inv(16)(p13.1q22) or t(16;16)(p13.1;q22) is an AML that usually shows monocytic and granulocytic differentiation and the presence of a characteristically abnormal eosinophilic component in the marrow [1]. The chromosomal rearrangements inv(16) or t(16;16), resulting in a CBFB/MYH11 fusion gene, are present in approximately 10% of all cases of de novo adult AML and are recognized as a good prognostic factor [2]. On the molecular level, the spectrum of CBFB/MYH11 fusion transcripts in AML with inv(16)/t(16;16) is heterogeneous and dependent on the exons of the CBFB and MYH11 genes that are fused. A total of 10 different CBFB/MYH11 transcripts (A-J subtypes) and other variants have been identified [3–6]. Of all CBFB/MYH11 positive cases, more than 80% have transcript type A, and around 5% each reveals transcripts D and E. Although the type A CBFB/MYH11 transcript shows the typical morphology and other classic characteristics of acute myelomonocytic leukemia with eosinophilia (AML-M4Eo), rare types of fusion transcripts are correlated with an atypical cytomorphology not primarily suggestive for the French-American-British (FAB) subtype of AML-M4Eo [3]. In this study, we report on a novel CBFB/MYH11 variant transcript with partial insertion of exon 6 of the CBFB gene using two commercially available reverse transcriptase-polymerase chain reaction (RT-PCR) kits.

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2. Materials and Methods

A 20-year-old Korean man was admitted to Severance Hospital of Yonsei University for further evaluation of pneumonia and hematologic malignancy. Initial complete blood count (CBC) showed a hemoglobin (Hb) level of 11.0 g/dL, a platelet count of 100,000/μL, and a WBC count of 6,960/μL with 6% neutrophils, 44% lymphocytes, 6% monocytes, 1% eosinophils, 1% myelocytes, 1% metamyelocytes, 4% atypical lymphocytes, and 37% blasts. The bone marrow biopsy showed 50-70% cellular marrow with markedly increased myeloblasts with maturation, consistent with an AML-M2 morphology (Fig. 1). Flow cytometry showed the blasts to be positive for CD13, CD33, CD45, and MPO, and negative for CD3, CD7, CD10, CD14, CD19, CD20, cCD22, CD79a, and TdT. The initial karyotype of this patient was 46,XY in all 21 cells analyzed (Fig. 1). FISH signals from PML/RARA, AML1/ETO, BCR/ABL, and MLL probes (Abbott Molecular/Vysis, Des Plaines, IL) were within reference ranges, whereas the CBFB/MYH11 FISH showed abnormal break apart signals (one green, one orange, and one fusion) (Fig. 2). The patient was diagnosed with AML, and treated with ara-C and idarubicin. One month after the initial diagnosis, a second bone marrow examination was performed, revealing slightly hypocellular marrow (30–50% cellularity) with complete remission (CR) (Fig. 1). He was still in remission state during the follow up duration (3 months). For further evaluation, multiplex gene rearrangement tests were performed with both a Hemavision kit (Bio-Rad Laboratories, Hercules, CA) and Seeplex Leukemia kit (Seegene, Seoul, Korea), in addition to sequencing analysis.

Initially, a multiplex RT-PCR assay was performed using a Hemavision kit (Bio-Rad Laboratories, Hercules, CA). According to the manufacturer’s guidelines, this kit is designed to detect 28 kinds of multiple rearrangements simultaneously, including more than 80 breakpoints or mRNA splice variants. Briefly, cDNA was synthesized from 1 μg of RNA using cDNA mix and reverse transcriptase at 37°C for 45 minutes. The first and second PCR consisted of eight parallel reactions for each patient sample. The PCR mixture was incubated at 95°C for 10 minutes to activate the i-StarTaq™ DNA polymerase (iNtRON BIOTECHNOLOGY, Seongsam, Korea). The first PCR consisted of 25 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 1 minute 30 seconds at 72°C. This was followed by nested-PCR consisting of 20 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 1 minute 30 seconds at 72°C. The presence of a specific band prompted a split-out PCR with the corresponding set of split-out primers.

A multiplex RT-PCR assay by dual-priming oligonucleotides (DPO) was subsequently performed with a Seeplex Leukemia CBFB/MYH11 kit (Seegene, Seoul, Korea), which is designed to detect most of types of CBFB/MYH11 fusion transcripts. The DPO method was previously described elsewhere [7–9]. Briefly, DPO is composed of
three regions; a longer 5’-segment, a shorter 3’-segment, and a poly(I) linker that bridges these 2 segments. In designing the DPO, the position of the 3’-segment was determined first, at a site where 6–12 bases had a 40–80% GC content. 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 25 minutes.

The purified PCR product was inserted into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA) according to the manufacturer’s specifications. Plasmid DNA was prepared using the plasmid Mini kit (Qiagen GmbH, Hilden, Germany). Sequencing was performed using a BigDye terminator v 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and the M13F/M13R primer on a DNA sequencer (3730XL; Applied Biosystems). Sequence analysis was done using the Ensembl database (http://www.ensembl.org/index.html).

3. Results

Multiplex RT-PCR screening with a Hemavision kit (BioRad Laboratories) showed unusual size bands of about 400 bp in lane 6 and 1100 bp in lane 7 in addition to the internal control band of size 911 bp for each lane (data not shown). Subsequent split-out PCR results revealed that these two bands were the same aberrant CBFB/MYH11 fusion transcript (Fig. 3). In addition, multiplex RT-PCR assay by the DPO method using a Seeplex Leukemia CBFB/MYH11 kit (Seegene) repeatedly showed a distinct band size of 1037 bp with the patient's marrow specimen (Fig. 3). The present results of this patient were not similar to CBFB/MYH11 subtypes reported previously. Finally, cloning and sequencing revealed that this band corresponded to novel breakpoints within both exon 6 of the CBFB (ENSG00000067955) gene and exon 28 of the MYH11 (ENSG00000133392) gene (Fig. 3). Detailed sequencing results are presented in Fig. 4.

Fig. 2. (a) Initial bone marrow aspiration (Wright-Giemsa, ×1000) showing several large abnormal myeloblasts with maturation. (b) Follow-up bone marrow examination (Wright-Giemsa, ×1000) demonstrating complete remission. (c) Fluorescence in situ hybridization (FISH) analysis of bone marrow cells using the CBFB (LSI CBFB Dual Color, Break Apart Probe; Abbott Molecular/Vysis) breakapart probe showing one green, one orange, and one fusion signal (initial diagnosis). (d) Follow-up FISH analysis using the CBFB (Abbott Molecular/Vysis) breakapart probe showing 2 normal fusion signals (one month later).
4. Discussion

There is increasing recognition of the importance of genetic events in the classification, therapy, and prognosis of AML. The new World Health Organization (WHO) classification system for AML and related myeloid neoplasms includes the following 7 entities: AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, therapy-related myeloid neoplasms, myeloid sarcoma, myeloid proliferations related to Down syndrome, blastic plasmacytoid dendritic cell neoplasms, and AML not otherwise specified [1]. Among these, AML associated with certain recurrent translocations such as t(8;21)(q22;q22), t(15;17) (q22;q12), and inv(16)(t(16;16)) are accompanied by a relatively favorable response to therapy and clinical behavior. Although fusion genes in various other hematologic malignancies also create alternative splicing or variant transcripts, the CBFB/MYH11 fusion gene makes the most complex and numerous transcripts (types A-J) in AML. As far as we know, these transcripts involve 2 CBFB breakpoints at nucleotides 399 or 495 and 8 MYH11 breakpoints: at nucleotides 1921 (types A and F, exon 33), 1708 (type B, exon 32), 1528 (type C, exon 31), 1201 (types D and G, exon 29), 994 (type E, exon 28), 1098 (type H, exon 28), 2134 (types I, exon 34), or 1306 (type J, exon 30) [3–6,10]. The type A transcript is the most commonly detected isoform in Western countries; however, type I was reported by Monma et al. [10] as a more frequent isoform in Japanese population.

This study is interesting from both a laboratory and clinical perspective. First, not only does this case show a partial insertion of exon 6 of the CBFB gene, but it also involves novel breakpoints within both exon 6 of the CBFB gene and exon 28 of the MYH11 gene. This creates a previously non-reported, new type (K-type) of the CBFB/MYH11...
fusion transcript. Secondly, our case did not show the typical findings in patients with AML-M4Eo. Initial bone marrow, chromosome, and flow cytometric analyses led us to diagnose this patient with AML-M2 on the basis of morphology and phenotypes. However, 2 kinds of commercially available multiplex RT-PCR analyses revealed the presence of a cryptic novel CBFB/MYH11 fusion transcript in our patient. Both multiplex RT-PCR kits in our study enable us to detect at least 8-9 kinds of known CBFB/MYH11 fusion transcripts. Therefore, we think that multiplex RT-PCR analysis is very useful for detecting complex or various fusion transcripts in AML patients with CBFB/MYH11 or other rearrangements.

5. Conclusions

We have described a novel CBFB/MYH11 fusion transcript that could be difficult to detect with conventional diagnostic methods. As previously reported by Schnittger et al. [3], rare fusion transcripts of CBFB/MYH11 are correlated with an atypical cytomorphology and other aberrant characteristics such as AML-M2 morphology or weak monocytic expression (low NSE activity or negativity for CD14). In such a situation, it could be helpful to use multiplex RT-PCR kits to detect rare variant fusion transcripts. In addition, sequence analysis of these atypical products should be routinely performed to diagnose atypical AML, to predict prognosis of these patients as well as to elucidate the molecular mechanism. To the best of our knowledge, this is the first report of a CBFB/MYH11 rearrangement revealing a complex transcript with partial insertion of exon 6 of the CBFB gene and novel breakpoints in both CBFB and MYH11 genes with atypical cytomorphism and other aberrant characteristics.

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