Case Report

New variant of acute promyelocytic leukemia with IRF2BP2–RARA fusion

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A
acute promyelocytic leukemia (APL) is an acute myeloid leukemia subtype characterized by promyelocytes with abnormal morphology, coagulopathy, specific chromosomal abnormalities, and unique response to retinoic acid treatment. Most APL patients carry t(15;17)(q22;q12), which results from the fusion of the promyelocytic leukemia (PML) and retinoic acid receptor α (RARA) genes. Consequently, the retinoid sensitivity of RARA is reduced, blocking myeloid differentiation. Other fusion partners of RARA have been found. Recently, interferon regulatory protein 2 binding protein 2 (IRF2BP2) emerged as a new RARA partner; this IRF2BP2–RARA fusion gene includes exon 2 of IRF2BP2 and intron 2 of RARA. Herein, we describe the second case of APL with the IRF2BP2–RARA fusion gene, in which novel breakpoints in IRF2BP2 and unique clinical characteristics were noted.

Case
A 68-year-old Japanese woman was admitted to Otemae Hospital (Osaka City, Japan) with pancytopenia in May 2012. Initial complete blood counts showed: hemoglobin, 8.2 g/dL; platelets, 48 × 10^9/L; and white blood cells, 1.65 × 10^9/L (including 1% promyelocytes). No disseminated intravascular coagulation (DIC) was detected. A bone marrow sample showed marked hypercellularity; 36.8% were promyelocytes with azurophilic granules and Auer rods (Fig. 1a). Flow cytometry revealed APL cells positive for cluster of differentiation (CD) 33, CD34, CD64, CD117, and human leukocyte antigen (HLA)-DR, and negative for CD2, CD7, CD13, and CD19. Fluorescence in situ hybridization (Fig. 1b) and RT-PCR failed to detect PML–RARA fusion, and chromosomal examination of leukemia cells showed X. Fluorescence in situ hybridization with dual fusion translocation probes detected no typical RARA split signals, but rather a small RARA signal and two large signals (46/100) (Fig. 1c). Accordingly, the patient was diagnosed with APL, and remission induction therapy was initiated with all-trans retinoic acid (ATRA; 45 mg/m^2/day). On day 12, promyelocytes in the peripheral blood increased to >10 × 10^9/L; therefore, we added idarubicin (9 mg/m^2/day from days 12 to 14) and cytosine arabinoside (75 mg/m^2/day from days 12 to 18). However, complete remission (CR) was not achieved. Re-induction therapy was initiated with gemtuzumab ozogamicin (GO) (9 mg/m^2) because of high CD33 expression. One month later, morphological examination and FISH of bone marrow aspirate showed hematological...
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(a) (b)

(c) (d)

1000 bp
500 bp

(e)

(f)

< IRF2BP2 >

Exon 1 Exon 2

Variant 1

1029

AAAA

Variant 2

1

RP1

48 bp

AAAA

< RARA >

Exon 1 Exon 2 Exon 3

AUG AAAA

< IRF2BP2–RARA >

(g)

< IRF2BP2 >

Exon 1 Exon 2

48 bp

< RARA >

Exon 1 Exon 2 Exon 3 Exon 4 Exon 5 Exon 6 Exon 7 Exon 8 Exon 9

AUG 48 bp

< IRF2BP2–RARA >
remission. In April 2013, the patient experienced molecular relapse as determined by an increase of Wilms tumor-1 mRNA in the peripheral blood. She was re-administered with GO (9 mg/m²), and remained in hematological remission until August 2013 when she developed syncpe. Magnetic resonance imaging revealed several enhanced regions in the right brain, and her cerebrospinal fluid (CSF) clearly contained APL cells. Because her level of consciousness was depressed despite high-dose methotrexate and cytarabine infusion, whole brain irradiation was undertaken. One month later, her neurological symptoms disappeared, and her CSF cytology revealed no APL cells. In February 2014, she developed bilateral leg weakness and walking difficulty. Lumbar magnetic resonance imaging showed enhancement in the conus and nerve roots, and APL cells were once again detected in her CSF. Craniospinal irradiation was undertaken, and her symptoms disappeared temporarily. However, she relapsed in August 2014 and died of APL.

After obtaining written informed consent and institutional review board approval, paired-end mRNA sequencing was carried out to identify the fusion gene partner of RARA. Consequently, we identified a chromosomal translocation between chr.1q42.3 and 17q21.2. This translocation gives rise to a putative fusion protein comprising exon 1 of IRF2BP2 and exons 3–9 of RARA. IRF2BP2 comprises two exons with an intron, and there are two splicing sites within IRF2BP2 exon 1. Accordingly, we generated primer pairs to amplify the cDNAs of IRF2BP2 (FP and RP1) and IRF2BP2–RARA (FP and RP2). This primer information is shown in Appendix S1. As predicted, two fragments were amplified by RT-PCR for both IRF2BP2 and IRF2BP2–RARA (Fig. 1d). The PCR product sequence corresponded well to the splicing sites (at the 1031 and 1079 bp positions of IRF2BP2 cDNA) (Fig. 1e). The RARA transcript started at the beginning of exon 3 (at 769 bp of RARA cDNA). Thus, our additional mRNA analysis clearly indicated that the IRF2BP2–RARA fusion proteins showed variant forms that were dependent on IRF2BP2 splicing. The transcript structures for IRF2BP2 and RARA, as well as IRF2BP2–RARA, are shown in Figure 1f. Taken together, the IRF2BP2–RARA gene is likely to be positioned between intron 1 of IRF2BP2 and intron 2 of RARA (Fig. 1g).

Discussion

To our knowledge, this is the second report of a novel APL variant with IRF2BP2-RARA; this case uniquely showed atypical morphological and clinical features with no DIC detected at admission.

Flow cytometry is a powerful ancillary tool for diagnosing APL. Classic APL cells with PML–RARA typically express mature myeloid markers, including CD13 and CD33, and are negative for CD34 and HLA-DR. Moreover, several reports have shown expression of CD34 and/or HLA-DR in a significant proportion of newly diagnosed APL patients. In our case, the APL cells were positive for CD34, CD33, CD4, CD117, and HLA-DR, and negative for CD2, CD7, CD13, and CD19.

Fluorescence in situ hybridization with dual fusion translocation probes is useful for detecting unknown rearrangements of the RARA gene. Herein, we failed to detect PML–RARA fusion; however, use of a RARA dual fusion translocation probe allowed us to detect a novel rearrangement of the RARA gene. Cases of APL with cryptic PML–RARA rearrangement that were negative by FISH and conventional karyotyping but positive by RT-PCR have been reported; these findings were caused by small cryptic insertions. Abnormal FISH and normal karyotyping also suggested small cryptic insertions in our case. A normal yellow signal indicated normal 17q while another indicated a small deletion in 17q; furthermore, an abnormal small yellow signal indicated a small insertion of RARA (Fig. 1).

The IRF2BP2–CDX-1 fusion gene has been reported in mesenchymal chondrosarcoma, and there is one previous report of IRF2BP2–RARA in APL. Yin et al. reported a case of APL with IRF2BP2–RARA that involved exon 2 of IRF2BP2 and intron 2 of RARA, with breakpoints at 1687 bp in IRF2BP2 and 41620 bp in RARA. The patient achieved CR with ATRA, arsenic trioxide, and GO; however, relapse occurred 2 months after consolidation therapy. Herein, IRF2BP2 showed distinct breakpoints, and two subtypes of IRF2BP2–RARA were noted. Our case differed in its atypical morphology and clinical course, as well as the lack of DIC on admission. Tissue factors and cancer procoagulant expressed in APL cells are associated with DIC; the lack of DIC in our case may be explained by the lower number of APL cells compared to that in the previously reported patient. Most patients with APL can achieve CR with ATRA with or without anthracycline, resulting in favorable clinical outcomes. In the previous IRF2BP2–RARA case, clinical sensitivity to ATRA was not properly evaluated because of combination therapy with arsenic trioxide and GO. Our patient did not respond to ATRA monotherapy. In the presence of pharmacological doses of concentrations of ATRA, transcriptional activation can be induced by dissociation of the co-repressor complex from PML–RARA as well as proteasome-dependent PML–RARA degradation. In cases with ligand binding domain mutations in the RARA gene,
ATRA fails to recognize RARA, and co-repressor dissociation does not occur, resulting in ATRA resistance. Other fusion genes such as PLZF–RARA and STAT5b–RARA also show ATRA resistance. The Nuclear receptor corepressor (N-CoR)/Silencing mediator for retinoid and thyroid hormone receptors (SMRT) co-repressor complex interaction with PLZF and STAT5b translocation is thought to be involved in this resistance. IRF2BP2 also interacts with N-CoR co-repressor proteins; we speculate that IRF2BP2–RARA is involved in ATRA resistance because of this.

In conclusion, we described a case of APL with IRF2BP2–RARA involving exon 1B of IRF2BP2. The patient had some peculiar clinical characteristics, such as no coagulopathy, a unique cell surface phenotype, and ATRA resistance. Although APL with IRF2BP2–RARA is rarely observed, its unique characteristics have clinical implications for the diagnosis and treatment of APL patients.

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Disclosure Statement

The authors have no conflict of interest.

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