

Case Report

New variant of acute promyelocytic leukemia with *IRF2BP2*–*RARA* fusionYoshimitsu Shimomura,¹ Hideki Mitsui,^{2,*} Yukiko Yamashita,² Tsuyoshi Kamae,² Akinori Kanai,³ Hirotaka Matsui,⁴ Tomohiko Ishibashi,⁵ Akira Tanimura,⁵ Hirohiko Shibayama,⁵ Kenji Oritani,⁵ Jun Kuyama² and Yuzuru Kanakura⁵

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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.⁽¹⁾ Consequently, the retinoid sensitivity of *RARA* is reduced, blocking myeloid differentiation. Other fusion partners of *RARA* have been found.^(2–9) 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*.⁽¹⁰⁾

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, $48 \times 10^9/L$; and white blood cells, $1.65 \times 10^9/L$

We present an acute promyelocytic leukemia (APL) patient with two subtypes of *IRF2BP2*–*RARA*, in which the *IRF2BP2* gene showed completely new breakpoints. Bone marrow examination revealed morphologic features indicative of APL. However, promyelocytic leukemia–*RARA* fusion was not detected. A paired-end mRNA sequencing followed by RT-PCR and direct sequencing revealed two types of fusion transcripts between exon 1B of *IRF2BP2* and exon 3 of *RARA*. The patient received all-trans retinoic acid and conventional chemotherapy, but showed resistance. This is the second report of *IRF2BP2* involvement in APL, and we describe various breakpoints for the *IRF2BP2*–*RARA* fusion gene.

(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/m²/day). On day 12, promyelocytes in the peripheral blood increased to $>10 \times 10^9/L$; therefore, we added idarubicin (9 mg/m²/day from days 12 to 14) and cytosine arabinoside (75 mg/m²/day from days 12 to 18). However, complete remission (CR) was not achieved. Re-induction therapy was initiated with gemtuzumab ozogamicin (GO) (9 mg/m²) because of high CD33 expression. One month later, morphological examination and FISH of bone marrow aspirate showed hematological

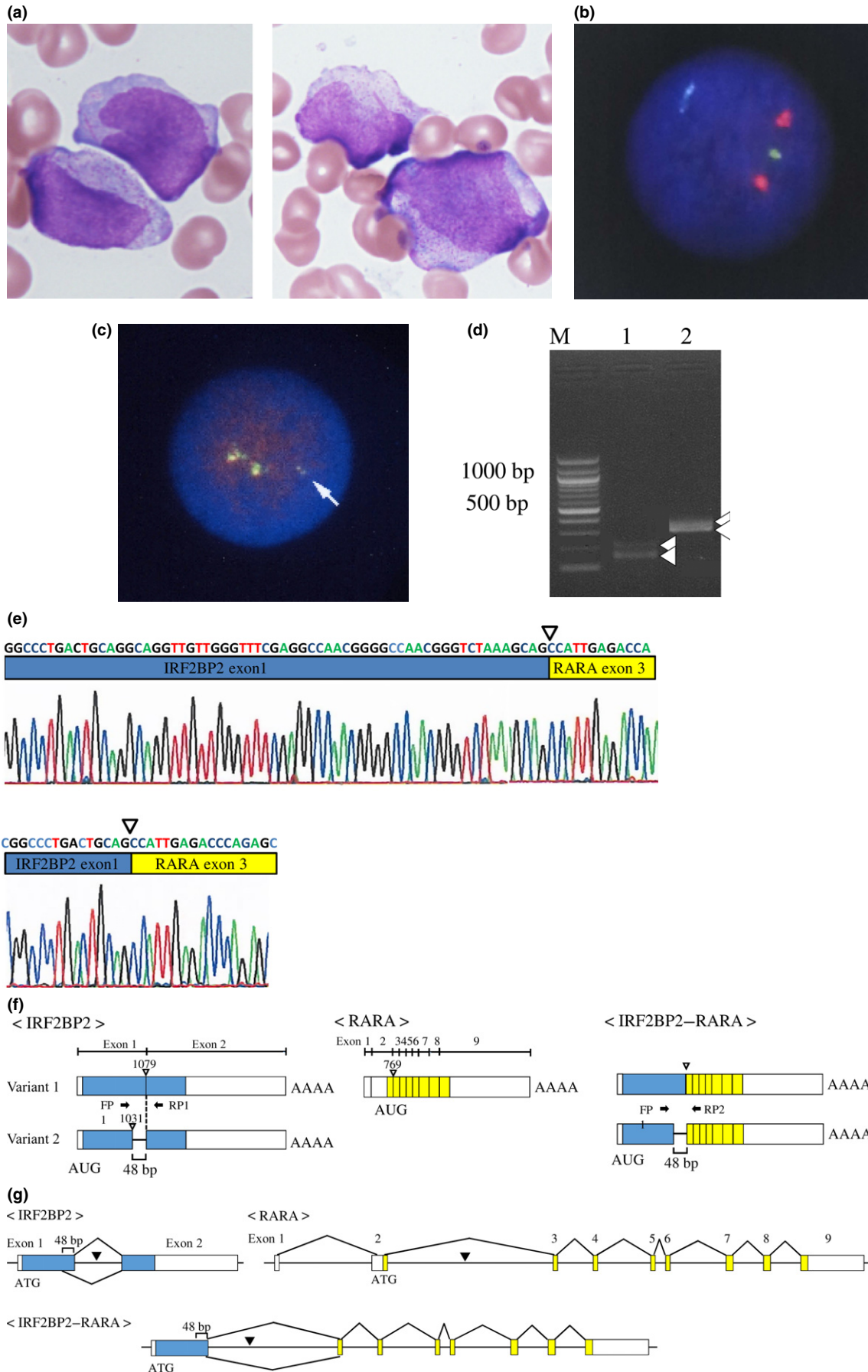


Fig. 1. Morphological, cytogenetic, and molecular features of a bone marrow sample from a 68-year-old Japanese woman with acute promyelocytic leukemia. (a) May–Giemsa staining. Promyelocytes with azurophilic granules and Auer rods are observed. (b) Interphase FISH using promyelocytic leukemia (*PML*) (15q22; green) and retinoic acid receptor α (*RARA*) (17q21; red) probes did not reveal a *PML*–*RARA* fusion signal (yellow). (c) Interphase FISH assay to detect *RARA* split signal (using 3'–*RARA* [green] and 5'–*RARA* [red] probes) showed no split signals but rather a small yellow signal (46/100; arrow). (d) mRNA expression of interferon regulatory factor 2 binding protein 2 (*IRF2BP2*) and *IRF2BP2*–*RARA*. Lane 1 shows the result of using two complementary oligonucleotide primers, FP1 and RP1. The upper detected band was predicted to be 206 bp and the lower band was predicted to be 158 bp; these bands were supposed to correspond to the expression of normal splice variants of *IRF2BP2* in the patient's bone marrow. Lane 2 shows the result of FP1 and RP2. The upper detected band was predicted to be 398 bp and the lower band was predicted to be 350 bp; these bands were supposed to correspond to the expression of the fusion variants of *IRF2BP2*–*RARA*. (e) Direct sequencing of the PCR products. The sequences of each PCR product obtained in (d) were determined. The results confirmed that this patient expressed two variants of *IRF2BP2* (data not shown), as well as the predicted fusion mRNAs of *IRF2BP2*–*RARA*; the upper and lower panels show the sequences of the fusion genes from *IRF2BP2* variants 1 and 2, respectively. (f) Schematic illustrations of the transcripts. The blue and yellow solid bars represent the coding regions of the *IRF2BP2* and *RARA* genes, respectively. *IRF2BP2* generates the transcript variants through alternative splicing. Variant 1 uses an alternate in-frame splice junction in the 3' coding region of exon 1, resulting in an mRNA transcript 48 bp longer than variant 2. The inverted triangles represent each predicted fusion point on the transcript. The *IRF2BP2*–*RARA* fusion is expected to generate two types of fusion mRNA depending on the *IRF2BP2* variants. In the longer type, base 1079 of the *IRF2BP2* mRNA is likely to be fused to base 769 of the *RARA* mRNA. In the shorter type, the base 1031 of the *IRF2BP2* mRNA is likely to be fused to base 769 of the *RARA* mRNA. (g) Overviews of genomic DNA of the *IRF2BP2*, *RARA*, and predicted *IRF2BP2*–*RARA* fusion genes.

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 syncope. Magnetic resonance images 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 sequences 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.^(11–13) In our case, the APL cells were positive for CD33, CD34, CD64, 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.⁽¹⁴⁾ 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;⁽¹⁵⁾ 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,⁽¹⁶⁾ and there is one previous report of *IRF2BP2*–*RARA* in APL.⁽¹⁰⁾ Yin *et al.*⁽¹⁰⁾ reported a case of APL with *IRF2BP2*–*RARA* that involved exon 2 of *IRF2BP2* and intron 2 of *RARA*, with breakpoints at positions 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.^(17–19) 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 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.⁽¹⁴⁾ Other fusion genes such as *PLZF*-*RARA*⁽⁵⁾ and *STAT5b*-*RARA*⁽²⁰⁾ 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;⁽²¹⁾ we speculated 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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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1 Primer information for amplification of cDNAs of *IRF2BP2* (FP and RP1) and *IRF2BP2*-*RARA* (FP and RP2).