Identification of a Novel Fusion Gene, IRF2BP2-RARA, in Acute Promyelocytic Leukemia

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Abstract
Acute promyelocytic leukemia (APL) is characterized by the fusion of retinoic acid receptor alpha (RARA) with promyelocytic leukemia (PML) or, rarely, other gene partners. This report presents a patient with APL with a novel fusion between RARA and the interferon regulatory factor 2 binding protein 2 (IRF2BP2) genes. A bone marrow examination in a 19-year-old woman who presented with ecchymoses and epistaxis showed morphologic and immunophenotypic features consistent with APL. PML oncogenic domain antibody was positive. Results of fluorescence in situ hybridization, conventional cytogenetics, reverse transcription–polymerase chain reaction (RT-PCR), and oligonucleotide microarray for PML-RARA and common APL variant translocations were negative. Next-generation RNA-sequencing analysis followed by RT-PCR and direct sequencing revealed distinct breakpoints within IRF2BP2 exon 2 and RARA intron 2. The patient received all-trans retinoic acid, arsenic, and gemtuzumab ozogamicin, and achieved complete remission. However, the disease relapsed 10 months later, 2 months after consolidation therapy. This is the first report showing involvement of IRF2BP2-RARA fusion in APL, and it expands the list of novel RARA partners identified in APL.

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Acute promyelocytic leukemia (APL) is commonly associated with t(15;17)(q24.1;q21.2), which results in the fusion of the promyelocytic leukemia (PML) gene at chromosome 15q24.1 with the retinoic acid receptor alpha (RARA) gene at chromosome 17q21.2.1 This fusion accounts for the disease response to all-trans retinoic acid (ATRA), and patients with APL usually have a favorable prognosis.1 However, although rare, RARA fuses with other genes in patients with APL, and reported partner genes include zinc finger and BTB domain containing 16 (ZBTB16, also known as promyelocytic leukemia zinc finger, PLZF),2,3 nucleophosmin (NPM1),4 nuclear mitotic apparatus (NUMA1),5 signal transducer and activator of transcription 5B (STAT5B),6 cAMP-dependent protein kinase type I-alpha regulatory subunit (PRKAR1A),7 FIP1-like 1 (FIPIL1),8 BCL6 corepressor (BCOR),9 and oligonucleotide/oligosaccharide-binding fold-containing 2A (OBFC2A).10 The identity of the partner gene is clinically important, because some RARA partner genes have been associated with resistance to ATRA therapy, in particular ZBTB16-RARA and STAT5B-RARA.3,6 This report presents a patient with APL for whom cytogenetic and molecular testing did not show evidence of t(15;17)(q24.1;q21.2)/PML-RARA, which led to the discovery of interferon regulatory factor 2 binding protein 2 (IRF2BP2-RARA), a novel gene partner for RARA.

Case Report
A 19-year-old woman presented with ecchymoses and epistaxis. Results of a CBC count showed a WBC count of 4.5 x 10^9/mL, hemoglobin level of 9.1 g/dL, and a platelet...
count of 29,000 x 10^3/mcL, with 28% promyelocytes. She had a prolonged prothrombin time (PT) and activated partial PT (aPTT) of 19.6 and 46.3 seconds, respectively, an elevated D-dimer level greater than 20 mcg/mL, and a decreased fibrinogen level of 60 mg/dL. Promyelocytes in the peripheral blood and disseminated intravascular coagulopathy were suggestive of APL. The patient was initiated on ATRA therapy. Her bone marrow was hypercellular (100%), with 59% promyelocytes, infrequent Auer rods, strong myeloperoxidase reactivity (cytochemistry), and a positive PML oncogenic domain (POD) test (Figure 1). Flow cytometry immunophenotyping showed an aberrant promyelocytic immunophenotype. Conventional cytogenetic analysis showed a diploid karyotype. Fluorescence in situ hybridization using a break-apart RARA probe was negative. Reverse transcription–polymerase chain reaction (RT-PCR) and oligonucleotide microarray did not detect any translocations commonly seen in APL including t(15;17)(q22;q21)/PML-RARA, t(5;17)(q35;q21)/NPM1-RARA, t(11;17)(q13;q21)/NUMA1-RARA, and t(11;17)(q23;q21)/ZBTB16-RARA. A screen for gene mutations detected a low-level mutation in NRAS (c.35G>A, p.Gly12Asp, <5%).

Based on the morphologic features and positive myeloperoxidase and POD tests, the patient was diagnosed with APL. Therapy with ATRA was continued, and arsenic trioxide (given on day 2) and gemtuzumab ozogamicin (1 dose on day 6) were added to the therapeutic regimen. The patient responded to treatment well; however, it took nearly 2 weeks for her PT, aPTT, and fibrinogen levels to return to normal, and her D-dimer level remained slightly elevated at 3.57 mcg/mL at last checkup, 11 months post-diagnosis of APL. She achieved complete molecular remission 5 weeks later and received 8 months of consolidation therapy with ATRA and arsenic trioxide. However, the patient experienced relapse 2 months after completion of consolidation therapy. She then received salvage therapy with ATRA, arsenic trioxide, and idarubicin, followed by haploidentical bone marrow transplantation. At the time of writing, she was experiencing complete remission 15 months after the initial diagnosis of APL.

Next-generation RNA-sequencing (RNA-seq) analysis was performed to identify the fusion gene partner. Library construction was performed using 700 ng of total RNA and the TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, CA). The library was selectively enriched by 12 cycles of PCR, followed by size selection, per the manufacturer’s protocol. The resulting size-fractionated library was sequenced using a 75–base pair (bp) paired-end reads protocol on an Illumina HiSeq 2000 Sequencing System. The resultant *.bcl files were converted to .FASTQ files using Illumina’s offline base calling software CASAVA (http://support.illumina.com/sequencing/sequencing_software/casava). After standard quality control using RNA-SeQC (http://www.broad institute.org/cancer/cga/rna-seqc), FASTQ files were processed using TopHat-Fusion (http://ccb.jhu.edu/software/tophat/index.shtml) to detect potential fusions. Supporting evidence (number of split reads, mate pairs, mate pairs with split ends) was manually examined to remove false-positive calls. RNA-seq data from bone marrow aspirate were analyzed for fusion transcripts involving RARA. This resulted in the identification of 87 sequence-unique mate-pair reads that defined a translocation joining intron 1 of IRF2BP2 gene on chromosome 1 to intron 2 of RARA gene on chromosome 17. Sixteen sequence-unique split-reads (spanning putative breakpoints) were identified that further confirmed and refined

Figure 1 Morphologic findings. (A) Bone marrow biopsy specimen showing sheets of leukemic cells with irregular nuclei and abundant eosinophilic cytoplasm (hematoxylin-eosin, original magnification x200). (B) Bone marrow aspirate smear showing promyelocytes with distinct cytoplasmic granules and infrequent Auer rods (Wright-Giemsa stain, original magnification x1000). (C) Promyelocytes are strongly positive for myeloperoxidase (original magnification x1000). (D) Promyelocytic oncogenic domain antibody shows that the promyelocytes are positive (original magnification x1000).
the IRF2BP2-RARA fusion. The data suggest that multiple transcripts are likely driven off the fusion. Because of the strength of the RNA-seq data and the involvement of RARA, a novel IRF2BP2-RARA fusion was likely involved in the pathogenesis of this case of APL (Supplemental Table 1, available online, in this article, at JNCCN.org).

RT-PCR and genomic DNA PCR was performed to detect the IRF2BP2-RARA fusion. Total RNA was reverse transcribed using random hexamers. PCR was performed using primers designed to amplify IRF2BP2-RARA fusion transcripts from cDNA and genomic DNA (Figure 2A, B). The PCR product was sequenced and analyzed using the EMBL-EBI multiple sequence alignment tool Clustal Omega (Hinxton, Cambridgeshire, UK). Amplicons were obtained of 310 bp and 323 bp, respectively (Figure 2C). Sanger sequencing of genomic DNA PCR products revealed that the IRF2BP2-RARA fusion involved exon 2 of IRF2BP2 and intron 2 of RARA with breakpoints at position 1687 bp in IRF2BP2 and 41,620 bp in RARA (Figure 2D, E). These findings confirm the fusion partners and breakpoints detected by RNA sequencing.

**Discussion**

IRF2BP2, located at chromosome 1q42.3, encodes a nuclear protein that contains an N-terminal zinc finger and a C-terminal RING finger domain that interacts specifically with the C-terminal transcriptional repression domain of IRF2. IRF2BP2 acts as a transcriptional corepressor and represses transactivation of nuclear factor of activated T cells (NFAT) that regulates genes involved in cell cycle, differentiation, and apoptosis. IRF2BP2 is also an ischemia-induced coactivator of vascular endothelial growth factor A expression, which may contribute to revascularization of ischemic muscle. IRF2BP2 is a direct target gene of TP53, and its overexpression inhibits apoptosis by impeding TP53-mediated transactivation of the TP21 and BAX genes. IRF2BP2 has been identified as an antiapoptotic factor in breast cancer cell lines, and a tumor-associated antigen in monoclonal gammopathy of undetermined significance. Whole transcriptome sequencing identified a novel IRF2BP2-CDX1 fusion as a result of t(1;5)(q42;q32) in a case of mesenchymal chondrosarcoma.

RARA functions by binding to retinoic acid response elements as a heterodimer with retinoid X receptor (RXR). This RARA-RXR complex is required for promyelocyte differentiation. Various X-RARA fusion proteins could have a dominant negative effect on wild-type RARA and X proteins, or form heterodimers sequestering RXR and recruiting corepressors and histone deacetylase complex to

**Figure 2** Molecular characterization of IRF2BP2 and RARA fusion. (A) Primer sequences for the detection of IRF2BP2-RARA fusion. Primers were designed in exonic and intronic regions of the IRF2BP2 and RARA genes to cover putative fusion sequences from cDNA and genomic DNA. To facilitate sequencing of PCR amplicons, primers were tagged with M13 universal sequences. (B) Primer design covering IRF2BP2 exon 2 and RARA exon 3 or intron 2 for detection of IRF2BP2-RARA fusion in cDNA and genomic DNA, respectively. (C) Results of reverse transcription–polymerase chain reaction (RT-PCR) and genomic PCR for detecting IRF2BP2-RARA fusion. RT-PCR using forward primer (FP) from IRF2BP2 exon 2 and reverse primer (RP) from RARA exon 3 gives rise to an amplicon of 310 base pairs (bp). Genomic PCR using FP from IRF2BP2 exon 2 and RP from RARA intron 2 gives rise to an amplicon of 323 bp. (D) Direct sequencing of genomic PCR products reveals an IRF2BP2-RARA fusion with a distinct breakpoint and part of sequences from IRF2BP2 exon 2 and RARA intron 2. (E) Alignments of genomic sequences of IRF2BP2 exon 2, IRF2BP2-RARA fusion, and RARA intron 2 using Clustal Omega show breakpoints in exon 2 of IRF2BP2 gene and intron 2 of RARA gene.

X receptor (RXR). This RARA-RXR complex is required for promyelocyte differentiation. Various X-RARA fusion proteins could have a dominant negative effect on wild-type RARA and X proteins, or form heterodimers sequestering RXR and recruiting corepressors and histone deacetylase complex to
repress genes implicated in myeloid differentiation. Similarly, IRF2BP2-RARA may promote leukemogenesis by serving as a dominant negative regulator of RARA and IRF2BP2. In the present case, the patient initially responded well to ATRA, arsenic trioxide, and gemtuzumab ozogamicin; therefore, APL with IRF2BP2-RARA seems to be sensitive to these drugs. However, early disease relapse suggests that APL with IRF2BP2-RARA may have a more aggressive clinical course and may require more intensive therapy.

**Conclusions**

This report presents a case of APL with a novel IRF2BP2-RARA fusion. The patient initially responded to ATRA, arsenic trioxide, and gemtuzumab ozogamicin; however, her disease relapsed shortly after completion of consolidation therapy, suggesting that the sensitivity to these drugs is limited and of short duration. This is the first report showing involvement of IRF2BP2 in APL, and it expands the list of novel RARA partners identified in APL. This case also shows the importance of morphologic examination to establish the diagnosis of APL based on the combination of cytologic features, strong myeloperoxidase reactivity, and a positive POD test, which led to evidence of a novel RARA fusion partner.

**Acknowledgments**

The authors wish to thank Dr. Roger Schultz from Signature Genomics Laboratories for his assistance with oligonucleotide microarray analysis.

**References**