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CORRESPONDENCE

Report of *IRF2BP1* as a novel partner of *RARA* in variant acute promyelocytic leukemia

To the Editor:

Acute promyelocytic leukemia (APL), characterized by the *PML*::*RARA* fusion gene, is highly sensitive to all-trans retinoic acid (ATRA) and arsenic trioxide (ATO). However, nearly 2% of APL patients are caused by forming rare fusions involving *RARA* or other members of the retinoic acid receptors (*RARB* or *RARG*), and a considerable proportion of them show resistance to ATRA and ATO and had poor outcomes.[1] Therefore, identifying rare pathological fusion genes has important clinical significance for APL diagnosis and therapy. Here, we report the *IRF2BP1* gene as a novel *RARA* fusion partner in a patient with morphological, immunophenotypical, and transcriptome features of APL but lacking *PML*::*RARA*.

A 51-year-old female patient was admitted because of chest tightness, shortness of breath, fever, without obvious cause. Blood tests showed a hemoglobin level of 67 g/L, white blood cell (WBC) count of 25.05 $\times$ 10^9/L with 80% abnormal immature cells, and platelet count of 53 $\times$ 10^9/L. The fibrinogen and D-dimer levels were 1.00 g/L (ref. 2.00–4.00 g/L) and 35.39 μg/mL (ref. 0.00–0.55 μg/mL), respectively. Prothrombin time and activated partial prothromboplastin time were 17.3 seconds (ref. 10.5–13.0 s) and 28.6 s (ref. 23–35 s), respectively. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was positive by antigen and nucleic acid test. Bone marrow (BM) morphological analysis revealed hypercellularity of myeloid cells, with 74% immature cells consisting of predominantly abnormal hypergranular promyelocytes with occasional auer rods (Figure 1A). Moreover, the blasts were strongly positive for peroxidase stain (Figure 1A). Flow cytometric immunophenotypic studies demonstrated that the blasts were positive for CD13, CD33, CD117, and cytoplasmic myeloperoxidase but negative for HLA-DR, CD34, CD38, CD15, CD14, and other T- or B- lymphoid-related markers (Figure 1B). The presumptive diagnosis in this patient was APL, and she received ATRA starting on the first day of admission.

However, dual-color dual-fusion fluorescence in situ hybridization (FISH) with a specific probe for *PML* and *RARA* failed to detect the *PML*::*RARA* fusion transcript in the BM sample (Figure S1A), FISH with the break-apart probes of *RARA* also showed no rearrangement of *RARA* (Figure S1B). Leukemia-related fusion genes, including *PML*::*RARA*, *NPM1*::*RARA*, and *ZBTB16*::*RARA*, were negative by reverse transcription PCR. To search for the potential fusion gene, we conducted whole transcriptome sequencing on BM samples. A validated fusion gene calling pipeline utilizing the Arriba software[2] reported negative results. However, upon manual investigation of the mapping reads of *RARA* exon3 using the Integrative Genomics Viewer inspector software, we discovered a wealth of chimeric sequencing reads involving *IRF2BP1* exon 1 and *RARA* exon 3, linked by a 9-bp fragment derived from *RARA* intron 2 (Figure S1C,D). Subsequent confirmation through reverse transcription PCR and Sanger sequencing (supplemental methods) validated the presence of the tandem chimeric transcript (Figure 1C,D). We speculate that it is due to the non-canonical 9 bp intervening fragments that routine bioinformatic pipelines report negative results. *IRF1BP1*, a gene characterized by a single exon, displays a breakpoint at position c.322 in the fusion transcript. The committed locus of the 9 bp exonized *RARA* intron 2 fragment lies 3717 bp upstream of exon 3, with the two adjacent bases at its 3′ end being GT. We hypothesize that this GT, in conjunction with the AG at the 5′ end of RARA exon 3, forms a paired derived splicing donor and splicing acceptor signal sequences. We validated the splicing of *IRF1BP1* c.322 with *RARA* intron 2 at the locus of 9 bp exonized sequence at the genomic level using PCR and Sanger sequencing analysis (Figure S1E,F). The *IRF2BP1*::*RARA* is expected to result in a fusion protein with 513 amino acids, which contains the zinc finger domain of IRF2BP1, and the DNA binding domain (DBD) and ligand binding domain (LBD) of RARA (Figure 1E). We also conducted a comparison of the gene expression pattern in this case with a consecutive cohort of 42 *PML*::*RARA*-positive APL cases,[2] 343 pathogenic fusion gene-negative AML cases, and 50 healthy controls using t-distributed stochastic neighbor embedding (t-SNE) analysis based on whole transcriptome sequencing data. The results indicated that this patient's gene expression profile clustered with *PML*::*RARA*-positive cases, distinctly separated from fusion gene-negative AML and controls (Figure 1F), indicating patient with *IRF2BP1*::*RARA* has the same transcriptome features of APL with *PML*::*RARA*.

We made a presumptive diagnosis of APL based on the morphological and immunophenotypical results. The patient was treated with 40 mg of ATRA per day from the first day of admission and added idamycin (IDA 20 mg day 2–4) to induce apoptosis (Figure 1G). WBC and immature cells in peripheral blood gradually decreased. After 7 days of treatment, leukemia cells decreased to 10%. The patient discontinued therapy and was discharged on December 27, 2022, because of the patchy density increase in both lungs, which showed significant progression of COVID-19 compared to the time of admission (Figure S2).

To date, 19 rare partners of *RARA* (*ZBTB16*, *NUMA*, *STAT5B*, *NPM1*, *IRF2BP2*, *TBLR1*, *FIPIL1*, *BCOR*, *STAT3*, *PRKAR1A*, *OBFC2A*, *GTF2I*, *FNDC3B*, *NUP98*, *TNRC18*, *HNRNPC*, *TTMV-ORF2*, *NAB2*, and *THRAP3*) have been reported.[1,3–5] It is the first report that *IRF2BP1*, a gene with one exon located on chromosome19, is a fusion partner of

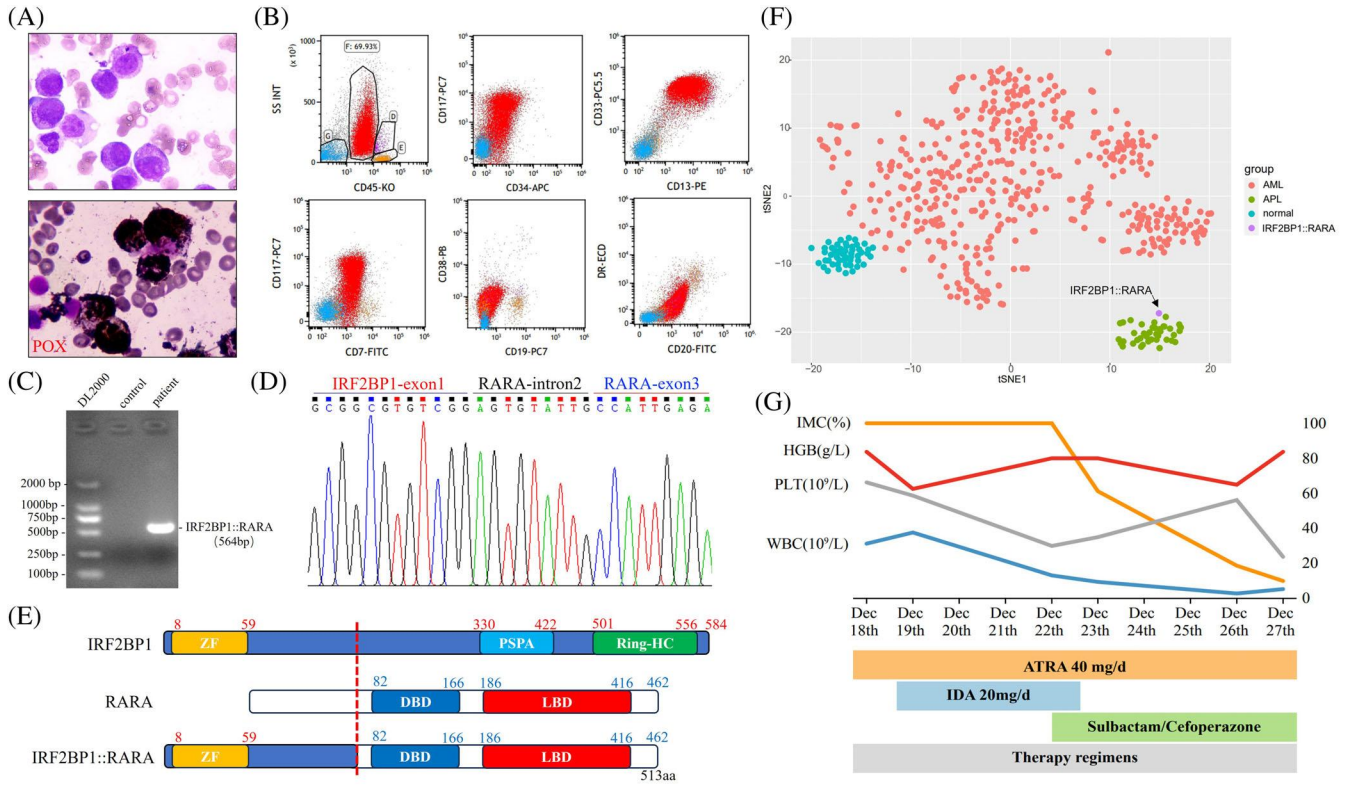

FIGURE 1 The main laboratory test results and clinical course of this patient. (A) Upper: Wright-Giemsa-stained bone marrow smear showed frequent promyelocytes with hypergranulated cytoplasm and invaginated nuclei (× 1000). Lower: myeloperoxidase stain showed a strong positive result (× 1000). (B) Flow cytometric analysis (CD45-dim blasts were gated) showed the blast cells expressed CD33, CD13, and CD117 and did not express CD34, CD7, CD38, HLA-DR, or other myeloid and lymphoid markers. (C) Electrophoresis of RT-PCR products showed the *IRF2BP1*::*RARA* fusion transcript in the patient's bone marrow sample. (D) Sanger sequencing analysis of the RT-PCR product. The fusion transcript was a fusion of IRF2BP1 exon 1 with RARA exon 3, linked by a 9-bp fragment derived from RARA intron 2. (E) Schematic diagram of the *IRF2BP1*::*RARA* fusion protein. ZF: zinc finger, PSPA: pneumococcal surface protein A, Ring-HC: variant RING-HC finger, DBD: DNA binding domain, LBD: ligand binding domain. The red dashed line indicates the freak and fusion sites. (F) Whole transcriptome expression pattern of the *IRF2BP1*::*RARA* case with a consecutive cohort of 42 *PML*::*RARA*-positive APL cases, 343 pathogenic fusion gene-negative AML cases, and 50 healthy controls by t-distributed stochastic neighbor embedding (t-SNE) analysis. (G) Clinical course of the patient. IMC: immature myeloid cell; HGB: hemoglobin; PLT: platelet; WBC: White blood cell.

RARA in acute myeloid leukemia with APL phenotype. IRF2BP1 is a member of the interferon regulatory factor 2 binding proteins (IRF2BP) family, which includes IRF2BP1, IRF2BP2, and IRF2BPL. IRF2BP family proteins act as E3 ligase containing an N-terminal zinc finger (ZF) and a C-terminal RING finger domain. Recent data suggest IRF2BP2 plays a role in different cellular functions, including apoptosis, survival, cell differentiation, angiogenesis, and immune response, but little is known about the biological function of the IRF2BP1 protein.[6] Some studies have reported the participation of *IRF2BP2* in the development of different kinds of cancer through gene fusion, Such as *IRF2BP2*::*CDX1* in mesenchymal chondrosarcomas, *IRF2BP2*::*NTRK1* in thyroid carcinoma, and *IRF2BP2*::*RARA* in APL. Indicating that IRF2BP family proteins are involved in the occurrence and development of tumors.

Although *IRF2BP1*::*RARA* fusion gene was identified by RNA sequencing and PCR, we did not detect the rearrangement of RARA using FISH, it is because the insertion was too small to visualize a split in FISH probes. Therefore, it is very valuable for multiple methods to identify RARA rearrangements in leukemia diagnosis, and RNA and genome sequencing technologies provide effective means for gene rearrangement tests.

Most variant APL cases without *PML*::*RARA* fusion were characterized by poor outcomes and resistance to ATRA and ATO. Some reports have shown that *IRF2BP2*::*RARA* positive APL is sensitive to ATRA, but likely susceptible to early relapse.[1] Our patient was treated with an indicating ATRA-based regimen after admission, and WBC and leukemia cells in peripheral blood gradually decreased following the treatment, indicating that the patient is sensitive to ATRA treatment. Unfortunately, COVID-19-associated pulmonary infection and coagulopathy comprise the main reason for the patient to give up treatment.

In summary, we identified a novel fusion gene *IRF2BP1*::*RARA* in a patient with APL. Although our patient gave up treatment because of COVID-19 infection, the short course of treatment can still show that patients with *IRF2BP1*::*RARA* may be sensitive to ATRA, which needs more clinical data support.

AUTHOR CONTRIBUTIONS

Zhanglin Zhang, Mei Jiang, and Xuemei Wang wrote the manuscript, Zhanglin Zhang, Mei Jiang, Xuemei Wang, Min Yu, Fei Li, and Hongxing Liu collected and analyzed the data. All authors revised the manuscript, read, and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

RNA-sequencing original data of the patient are available at GSA (https://ngdc.cncb.ac.cn/) under accession number HRA008380. For all the original data, please contact Zhanglin Zhang (ndyfy02270@ncu.edu.cn) or Hongxing Liu (starliu@pku.edu.cn).

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REFERENCES

1. Guarnera L, Ottone T, Fabiani E, et al. Atypical rearrangements in APL-like acute myeloid leukemias: molecular characterization and prognosis. *Front Oncol*. 2022;12:871590.
2. Chen X, Wang F, Zhang Y, et al. Fusion gene map of acute leukemia revealed by transcriptome sequencing of a consecutive cohort of 1000 cases in a single center. *Blood Cancer J*. 2021;11(6):112.
3. Ng Liet Hing M, Ryland GL, Nguyen T, et al. Variant acute promyelocytic leukaemia with novel NAB2::RARA fusion shows clinical all-trans retinoic acid and arsenic trioxide sensitivity. *Br J Haematol*. 2023;202(4):893-896.
4. Sala-Torra O, Beppu LW, Abukar FA, Radich JP, Yeung CCS. TTMV-RARA fusion as a recurrent cause of AML with APL characteristics. *Blood Adv*. 2022;6(12):3590-3592.
5. Song B, Wang X, Kong X, et al. Clinical response to Venetoclax and decitabine in acute promyelocytic leukemia with a novel RARA-THRAP3 fusion: a case report. *Front Oncol*. 2022;12:828852.
6. Pastor TP, Peixoto BC, Viola JPB. The transcriptional co-factor IRF2BP2: a new player in tumor development and microenvironment. *Front Cell Dev Biol*. 2021;9:655307.

SUPPORTING INFORMATION

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