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ORIGINAL ARTICLE

Identification of a novel fusion gene, RARA::ANKRD34C, in acute promyelocytic leukemia

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Abstract

Acute promyelocytic leukemia (APL) is a specific subtype of acute myeloid leukemia that is distinguished by the chromosomal translocation t(15;17)(q24;q21), which leads to the fusion of the promyelocytic leukemia (PML) gene with the retinoic acid receptor alpha (RARA). Recently, we identified a novel fusion gene in APL, RARA::ankyrin repeat domain 34C (ANKRD34C), identified its functions by morphological, cytogenetic, molecular biological and multiplex fluorescence in situ hybridization analyses, and demonstrated the potential therapeutic effect clinically and experimentally of all-trans retinoic acid (ATRA); the findings have important implications for the diagnosis and treatment of atypical APL.

Keywords Acute promyelocytic leukemia · ANKRD34C · RARA · PML::RARA

Acute promyelocytic leukemia (APL) is a specific subtype of acute myeloid leukemia distinguished cytogenetically by the chromosomal translocation t(15;17)(q24;q21), resulting in the promyelocytic leukemia-retinoic acid receptor α fusion gene. APL, with hyperfibrinolysis and disseminated intravascular coagulation as its common clinical manifestations, can result in severe bleeding tendencies [1–3]. Since the advent of arsenic trioxide (ATO) in the early 1990s and the combined therapeutic application of all-trans retinoic acid (ATRA) with ATO, APL has become one of the most potentially curable leukemias [4]. In addition to the typical PML::RARA fusion gene, other variant fusion gene loci, such as t(11;17)(q23;q21) leading to PLZF::RARA fusion and t(5;17)(q35;q21) leading to NPM::RARA fusion, can also infrequently lead to APL [5–7]. The discovery of these variant fusion genes is of great significance for the precise diagnosis and treatment of APL.

A 30-year-old man was admitted to the hospital in February 2020 because of a history of skin petechiae for more than 1 month and gingival bleeding for 7 days. Blood tests exhibited a white blood cell (WBC) count of 2.1×10^9/L, a hemoglobin (HB) concentration of 121 g/L, and a platelet (PLT) count of 8×10^9/L. The fibrinogen (Fib) concentration was 0.4 g/L (reference, 2.00–4.00 g/L). The prothrombin time (PT) was 15.3 s (reference, 10–15 s), and the partial thromboplastin time (APTT) was 28.5 s (reference, 25–31.3 s). The D2-dimer (D-D) was 20.89 mg/L (reference, <5 s), and the patient was diagnosed with disseminated intravascular coagulation (DIC). A bone marrow smear showed abnormal hypergranular promyelocytes accounting for 86% of bone marrow cells, consistent with AML-M3a (Fig. 1A). Flow cytometry showed that the blasts were positive for CD33, CD13, CD117, CD64 and CD38; partially positive for CD19, CD56, CD7 and cMPO; but negative for cCD3, cTDT, cCD79a, cCD22, etc. (Fig. 1B). Therefore, a combination of ATRA (20 mg/m^2, Tid, Days 1–28) and ATO (10 mg/kg, Qd, Days 1–28) was given. A karyotype of 46, XY, t(1;3)(q12;p24), t(4;8)(p16;p12), t(15;17;22)(q24;q21;q13)[19]/46,XY[1] was identified, using the RHG banding technique (Fig. 1C). FISH analysis of bone marrow specimens obtained at diagnosis using a PML::RARA dual-color, dual-fusion probe revealed PML::RARA fusion, and a diagnosis

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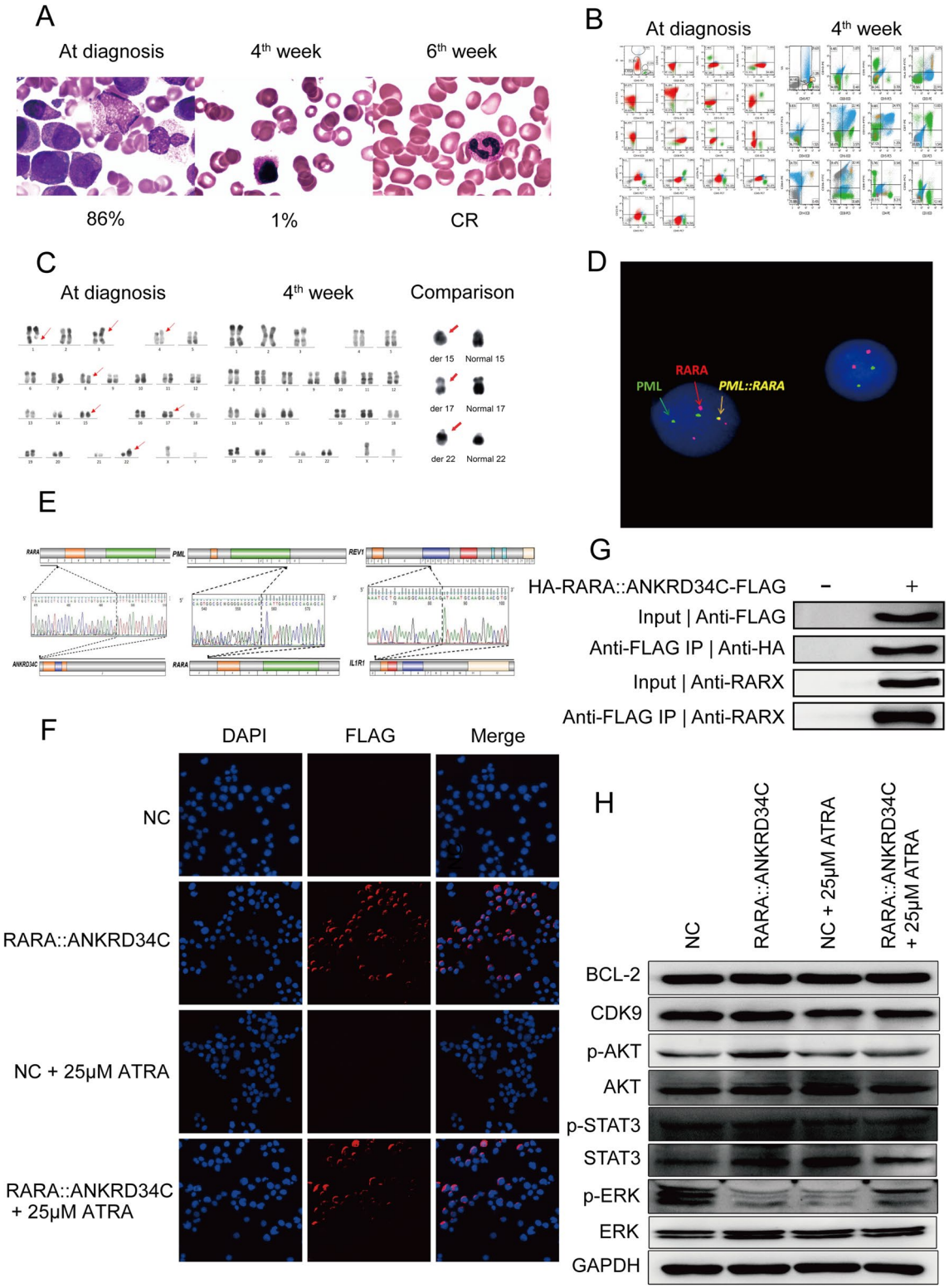

of AML-M3a (FAB)/APL was established (Fig. 1D). The rate of fusion signals was 92%, containing three red, two green, and one yellow fluorescent signals, showing that there may be a three-way translocation. Whole-transcriptome (mRNA) sequencing was performed to identify whether there was an abnormal rearrangement of the RARA gene, and the new fusion gene RARA::ANKRD34C, with ANKRD34C located on human chromosome 15q25.1, was identified. Three fusion genes were detected: PML::RARA, RARA::ANKRD34C, and REV1::IL1R1. PML::RARA

◂**Fig. 1** Molecular characterization, cellular localization, and transcriptional effects of the RARA::ANKRD34C fusion gene and dimerization of the RARA::ANKRD34C fusion protein. (**A**) The bone marrow smear showed abnormal hypergranular promyelocytes, original magnification 1000×. (**B**) Flow cytometric immunophenotype analysis. (**C**) A karyotype of 46,XY,t(1;3)(q12;p24),t(4;8)(p16;p12),t(15;17;22)(q24;q21;q13)[19]/46,XY[1] was identified, using the RHG banding technique. After treatment, the karyotype became normal on 20 metaphases. (**D**) Interphase FISH using a dual-color, dual-fusion translocation probe from Wuhan HealthCare Biotechnology Co., Ltd., revealed the fusion gene PML::RARA and clarified the diagnosis of APL. (**E**) Partial nucleotide sequences around the junctions of the RARA::ANKRD34C fusion transcript were formed by recombination of RARA exon 2 and ANKRD34C exon 2. Partial nucleotide sequences around the junctions of the PML::RARA fusion transcript were formed by recombination of PML exon 6 and RARA exon 3. Partial nucleotide sequences around the junctions of the REV1::IL1R1 fusion transcript were formed by recombination of REV1 exon 8 and IL1R1 exon 4. (**F**) Immunofluorescence analysis of normal peripheral blood monocytes (NCs), NCs with RARA::ANKRD34C, NCs treated with 25 μM ATRA and NCs with RARA::ANKRD34C treated with 25 μM ATRA. An anti-FLAG antibody was used as the primary antibody, and 4′,6-diamidino-2-phenylindole (DAPI) was used for nuclear staining. (**G**) Homodimerization of the RARA::ANKRD34C fusion protein and heterodimerization of the RARA::ANKRD34C fusion protein and RXRA protein were identified by coimmunoprecipitation. (**H**) Immunoblot analysis of the protein levels of BCL-2, CDK9, AKT, p-AKT, STAT3, p-STAT3, ERK, and P-ERK was accomplished on normal peripheral blood NCs, NCs with RARA::ANKRD34C, NCs treated with 25 μM ATRA and NCs with RARA::ANKRD34C treated with 25 μM ATRA

fusion gene was formed by recombination of PML exon 6 and RARA exon 3. RARA::ANKRD34C fusion gene was formed by recombination of ANKRD34C exon 2 and RARA exon 2. REV1::IL1R1 fusion gene was formed by recombination of REV1 exon 8 and IL1R1 exon 4. Among the three, RARA plays a great role in the pathogenesis of leukemia and PML::RARA is a common non-novel fusion gene, so we focused on the RARA::ANKRD34C fusion gene and did not specifically describe the others. Based on FISH analysis, chromosomal karyotyping analysis and mRNA sequencing, the observed hybridization pattern illustrated that 17q21-qter translocated to 15q24, 15q24-qter translocated to 22q13, and 22q13-ter connected to 17q21, so the PML::RARA fusion gene was formed (Fig. 1D). Therefore, a yellow fusion signal appeared on chromosome 15 with 15q24 composed of der(22)t(22,15)(22q13,15q24). A red fusion signal appeared on the derived chromosome 22 with 22q13 composed of der(17)t(17,22)(17q21,22q13). A green fusion signal appeared on the derived chromosome 17 with 17q21 composed of der(15)t(15,17)(15q24,17q21). After comparing the constituent of the new fusion gene with those of other genes in the NCBI database, we confirmed that the fusion gene was formed by recombination of RARA exon 2 and ANKRD34C exon 2 (Fig. 1E). Bone marrow puncture was repeated after 4 weeks, and the smear showed decreased bone marrow hyperplasia with 1% abnormal hypergranular promyelocytes (Fig. 1A). The karyotype became normal on 20 metaphases (Fig. 1C). The patient achieved cytologic remission. Therefore, ATRA was still administered after the patient was discharged from the hospital. A bone marrow smear in the 6th week showed decreased bone marrow hyperplasia and a significantly increased proportion of segmented neutrophils (Fig. 1A). The patient was in a low-risk group according to the MICM classification [8, 9]. Therefore, consolidation therapy with the IA regimen was administered in accordance with the NCCN guidelines, consisting of idarubicin (IDA) (8 mg/m^2/d, Days 1–3) and cytarabine Ara-C (100 mg/m^2/d, Days 1–5). The patient completed three consolidation therapy sessions according to this chemotherapeutic regimen. We used real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) of fusion gene PML::RARA transcripts for residual disease detection in APL, during which all repeated bone marrow punctures indicated that PML::RARA transcripts were "negative", revealing he was in molecular biological remission. Then, ATRA and ATO were used for maintenance therapy. The last follow-up of the patient was conducted on September 20, 2022.

To explore whether this novel fusion gene causes APL, we conducted a series of functional experiments. We expressed the labeled fusion protein in peripheral blood monocytes (NCs), and immunofluorescence analysis showed that the RARA::ANKRD34C fusion gene exhibited predominantly perinuclear localization. We also found that the expression of the RARA::ANKRD34C fusion gene was decreased in the presence of ATRA (Fig. 1F).

It has been reported that the key molecular pathogenic etiology of APL is chaperone gene-enhanced retinoic acid receptor dimerization. In normal cells, heterodimers of retinoic acid receptor α (RARA) and retinoic X receptor α (RXRA) can bind to retinoic acid response elements and mediate transcription. PML forms homodimers with RARA, and these homodimers compete with RARA for retinoic acid response elements and inhibit the transcriptional activation of target genes related to hematopoietic differentiation, blocking granulocyte differentiation [10–12]. Therefore, to verify whether RARA::ANKRD34C can compose homodimers and heterodimers, coimmunoprecipitation assays were conducted in peripheral blood monocytes. Haemagglutinin (HA)-tagged RARA::ANKRD34C was coimmunoprecipitated by FLAG-tagged RARA::ANKRD34C, and RXRA was coimmunoprecipitated by FLAG-tagged RARA::ANKRD34C (Fig. 1G), indicating that RARA::ANKRD34C can self-associate to form homodimers and associate with the RXRA protein to form heterodimers.

An empty vector as well as the HA-tagged and FLAG-tagged RARA::ANKRD34C vectors were introduced into peripheral blood monocytes (PBMCs), and some downstream targets were investigated [13–18]. Immunoblotting confirmed that the level of p-ERK was decreased by RARA::ANKRD34C expression, while the level of p-AKT was increased, and the level of p-STAT3 did not change significantly. The results suggested that the RARA::ANKRD34C fusion gene could probably enhance the proliferation ability of leukemia cells by activating antiapoptotic programs as well as AKT, a key molecule in the PI3K-AKT signaling pathway [19, 20]. In addition, immunoblotting showed that after treatment with ATRA, the level of p-ERK was increased, the levels of CDK9 and p-AKT were decreased, and the level of p-STAT3 remained unchanged, indicating that ATRA might inhibit signal transduction induced by RARA::ANKRD34C in acute promyelocytes (Fig. 1H).

In conclusion, we identified RARA::ANKRD34C as a novel RARA fusion gene that was closely associated with the pathogenesis and development of APL. This study demonstrated that multiple detection techniques can be used to verify the presence of the RARA::ANKRD34C fusion gene, explore pathogenesis, and therefore, provide experimental and theoretical support for further classification and precise therapy of APL.

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Author contributions Ying Du, Yonggong Yang, Lanxin Chen, Miaoxin Peng, and Peipei Xu conceived and designed the study. Yue Chen, Mengge Pan, Yiran Fang, and Zhenyu Liu analyzed the experimental data. Yue Chen, Mengge Pan, and Zhenyu Liu drafted the manuscript. All authors approved the final version of the manuscript.

Data availability The data of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval All participating sites received Institutional Review Board or ethics committees' approval for the protocol. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008(5).

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors report there are no competing interests to declare.

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