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Case Report

Identification of novel NUP98::RARA fusion transcripts in acute promyelocytic leukemia with i(17)(q10) abnormality

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Abstract: Acute promyelocytic leukemia (APL) is one subtype of acute myeloid leukemia (AML) primarily associated with the typical fusion gene PML::RARA/t(15;17). A small percentage of APL cases are caused by atypical gene transcript variants lacking the PML::RARA. We report one case with two novel NUP98::RARA fusion transcripts in APL lacking the fusion gene PML::RARA/t(15;17). These NUP98::RARA fusion transcripts were identified using next-generation sequencing (NGS), which were confirmed by Sanger sequencing. One of the transcripts differs from the previously reported transcript in terms of break sites and transcript length, which identified as subtype of NUP98::RARA fusion transcript. The other one is the same as previously reported, demonstrating reproducible abnormality of this fusion gene. The patient was treated with all-trans retinoic acid (ATRA), realgar-Indigo naturalis formula (RIF) and chemotherapy. According to the published paper, this is the second report of NUP98::RARA fusion transcript in APL. It is also the first variant APL with der(11)(p15)t(11;17)(p15;q21) and i(17)(q10) chromosome abnormalities. Therefore, we compared and summarized these two cases.

Keywords: APL, NUP98::RARA, i(17)(q10), t(11;17)(p15;q21)

Introduction

Acute promyelocytic leukemia (APL) is a distinct type of AML classified as the AML-M3 by the FAB. It represents 5-8% of AML cases and is characterized by the fusion gene involving retinoic acid receptor a (RARA) [1]. The majority of APL cases exhibit the PML::RARA/t(15;17)(q24;q21) fusion gene, while a proportion of APL cases, known as variant APL, lack this fusion gene and instead present with alternative genetic abnormalities such as PLZF::RARA/t(11;17)(q23;q12),TBLR19::RARA/t(3;17)(q26;q21), NPM-RARA/t(5;17)(q35;q12) [2-5]. A uncommon variant APL expressing the NUP98::RARA fusion gene with normal karyotype has also been reported previously [6].

Previous reports have described APL with chromosome i(17)(q10) abnormality, it appears to be accompanied by PML::RARA/t(15;17) [7-9]. The extreme rarity of NUP98::RARA positive APL with concurrent i(17)(q10) chromosomal abnormality severely limits both diagnostic experience and therapeutic decision-making for this variant, underscoring the critical need for expanded clinical investigations and molecular characterization studies. Herein we present the first case of variant APL with the NUP98::RARA fusion gene (t(11;17)(p15;q21)) and chromosome i(17)(q10) abnormality.

Case report

In October 2023, a 70-year-old male was admitted due to ecchymosis and a fever for more than 3 days. The blood test results were as follows: white blood cell (WBC) count 122.92×10^9/L (differential: neutrophils 5.0%, lymphocytes 15.0%, monocytes 5.0%, eosinophils

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0.0%, basophils 0.0%, immature cell 75%), hemoglobin (Hb) 101 g/L, and platelet (PLT) count 20×10^9/L. The C-reactive protein (CRP) concentration was 110.76 mg/L (ref.: 0-8.00 mg/L), the D-dimer concentration was 65692 μg/L (ref.: 0-700 μg/g), the activated partial thromboplastin time (APTT) was 27.8 s (ref.: 23.9-33.5 s), the thrombin time (TT) was 15.6 s (ref.: 14.5-21.5 s), the prothrombin time (PT) was 14.9 s (ref.: 10.0-13.5 s), and the fibrinogen (Fg) concentration was 1.88 g/L (ref.: 2.00-4.00 g/L).

The bone marrow (BM) smear showed hypercellularity, with 82% promyelocytes (**Figure 1A**). The majority of abnormal promyelocytes were round with full cytoplasm with purple-red azurophilic granules (partially), non-lobulated nuclei and nucleoli, but lacked Auer Rods. Flow cytometry revealed a group of abnormal cells that expressed CD117, CD34 (partially), CD33, CD13, CD38 (dim), CD56 and CD123. However, these cells lacked HLA-DR, CD4, CD16, CD11b, CD14 and CD15. Reverse transcription polymerase chain reaction (RT-PCR) was used to test the 42 common fusion genes, but all of the fusion genes were negative, including PML::RARA, PLZF::RARA, NUMA1::RARA, SATAT5b::RARA, F1P1L1::RARA, PRKAR1A::RARA and NPM::RARA. The PML::RARA fusion gene was detected as negative by fluorescence in situ hybridization (FISH) using a PML::RARA dual-color dual-fusion probe, but the RARA gene was duplicated and rearranged using RARA break-apart probe (**Figure 1B**, **1C**).

We collected a BM sample from this patient for mRNA sequencing (next-generation sequencing, NGS) (Illumina NovaSeq 5000). The read length of RNA sequencing was 151 bp and the data was analyzed by Arriba. NGS showed NUP98::RARA fusion gene with ruptured sites (11:3759570: -, 17:38496727: +) and (11:3765739: -, 17:38504568: +) (**Figure 1D**, **1E**), which revealed two transcripts of NUP98::RARA fusion gene (**Figure 2D**). To confirm these 2 NUP98::RARA fusion transcripts we designed 2 pairs of primers, F: 5'-TTGTGTTTCATCAGAAGACTTGG-3', R: 5'-GTTAAAGGGCGAGTCTGTGC-3' and F: 5'-TTGGAGGGCCTCTTGGTACA-3'; R: 5'-AGCAAGGCTTGTAGATGCGG-3', which were used for polymerase chain reaction (PCR) of cDNA. The PCR products (184 bp and 180 bp) were analyzed by gel electrophoresis (**Figure 2A**) and Sanger sequencing (**Figure 2B**, **2C**). The karyotype analysis (R-band) showed a result of 46,XY,der(11)(p15)t(11;17)(p15;q21), i(17)(q10) [20] based on the results of NGS and FISH (**Figure 1F**).

According to the results of bone marrow smear and FISH, a diagnosis of APL was made. The patient was started on hydroxycarbamide (1 g, TID) treatment to reduce WBC and all-trans retinoic acid (ATRA, 20 mg, BID) treatment to induce cell differentiation. Treatment with the oral arsenic realgar-Indigo naturalis formula (RIF, 1.35 g, TID) and (HHT, 2 mg, QD) was initiated for apoptosis induction and leukocytosis reduction. Unfortunately, this patient experienced cerebral hemorrhage with cerebral infarction due to a low PLT (18×10^9/L) and died two days later.

Discussion

In this study, we have identified the NUP98::RARA fusion gene and chromosome i(17)(q10) abnormality in APL. Variant APL usually presents with similar clinical and morphological features to those of typical APL, marking it challenging to distinguish variant forms from typical APL. Prompt diagnosis and treatment are crucial for variant APL patients.

In typical APL, most patients tested positive for the PML::RARA/t(15;17) fusion gene [10, 11]. The identification of novel fusion genes and genetic abnormalities in APL, while technically challenging, holds critical implications for therapeutic decision-making and patient survival. Although the RT-PCR and FISH remain cornerstone methodologies for detecting APL-related genetic alterations, the unavailability of target-specific primers and probes poses substantial technical barriers to characterizing rare/atypical fusion variants or cryptic chromosomal abnormalities. In our study, we identified the NUP98::RARA fusion by NGS, and confirmed it through RT-PCR and Sanger sequencing. The result showed negative for PML::RARA fusion gene by FISH using a PML::RARA dual-color dual-fusion probe, which showed 4 red signals and 2 green signals. It indicated that the RARA gene may duplicated or ruptured. The result showed RARA rearrangement positive by FISH using an RARA break-apart probe, which showed 1 green and 3 yellow signals. These results revealed that the RARA gene had three

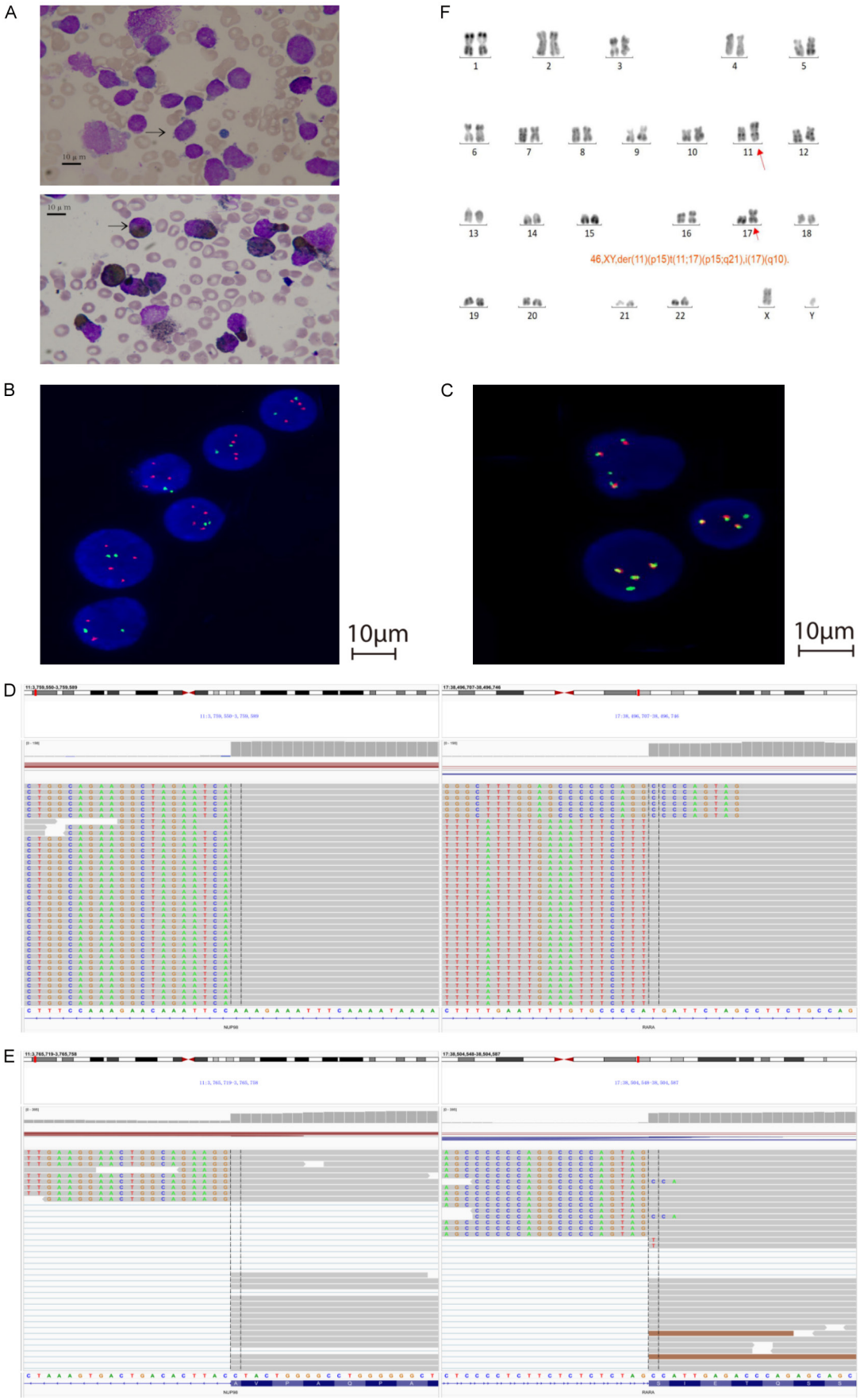
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Figure 1. Morphology, FISH, mRNA sequencing and karyotype of patient's myeloid blasts. A. Bone marrow smear: APL with large promyelocytes, full cytoplasm with purple-red azurophilic granules (partially), non-lobulated nuclei and nucleoli, and few Auer rods (top. Wright-Giemsa, ×1000); The peroxidase stain shows strong positivity (bottom, ×1000). B. FISH showed 4 red and 2 green signals using PML::RARA dual-color dual-fusion probe, PML::RARA was negative (×1000). C. FISH showed 3 yellow signals and 1 green signal using RARA break-apart probe, RARA rearrangement was positive(×1000). D. NGS of NUP98::RARA fusion gene with ruptured sites: (11: 3759570: -, 17: 38496727: +). E. NGS of NUP98::RARA fusion gene with ruptured sites (11: 3765739: -, 17: 38504568: +). F. R-banding karyotype analysis: 46,XY,der(11)(p15)t(11;17)(p15;q21),i(17)(q10).

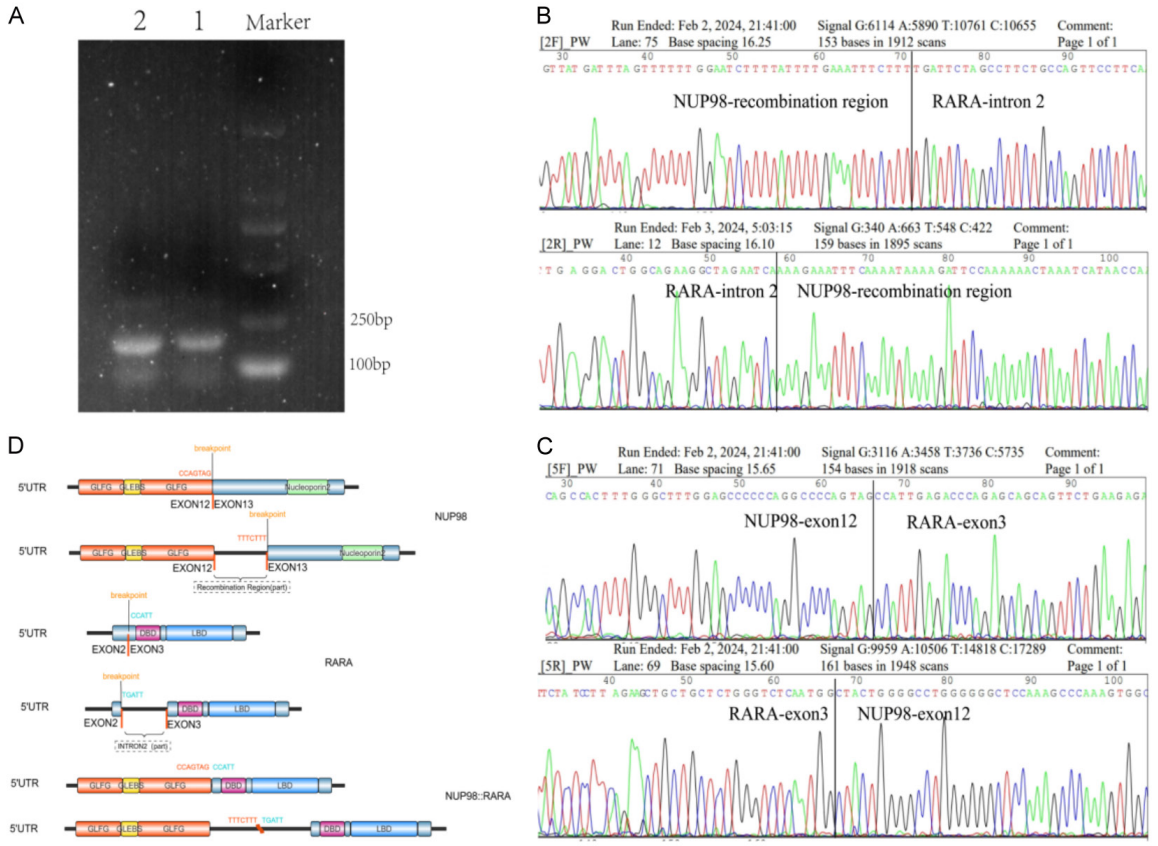

Figure 2. Gel electrophoresis and Sanger sequencing of PCR products, pattern of transcripts. (A) Gel electrophoresis of PCR products: 1: transcript of NUP98::RARA with a breakpoint in the recombination region of NUP98; 2: transcript of NUP98::RARA with a breakpoint in exon 12 of NUP98. (B, C) Sanger sequencing: two NUP98::RARA fusion transcripts, the NUP98::RARA transcript 1 with a breakpoint in the recombination region of NUP98 (B); the NUP98::RARA transcript 2 with a breakpoint in exon 12 of NUP98 (C). Both of the transcripts were bidirectionally sequenced and confirmed via BLAST. (D) NUP98::RARA transcripts: breakpoints in exon 12 and recombination region of the NUP98; breakpoints in exon 3 and intron 2 of the RARA; ligand-binding domain (LBD); DNA-binding domain (DBD); 5'-untranslated region (5'-UTR); glycine-leucine-phenylalanine-glycine (GLFG); Gle2/Rae1-binding sequence (GLEBS).

copies and one RARA gene segment translocation. NGS showed rearrangements of t(11;17) on chromosome 11 indicating the presence of NUP98::RARA fusion gene. In Karyotype analysis, we found a derivative chromosome 11 and a chromosome i(17)(q10). According to the results of FISH and NGS, this patient must be a new variant APL with NUP98::RARA fusion and i(17)(q10) abnormality. Indeed, we have identified distinct cell morphological characteristics in NUP98::RARA fusion APL compared to classic gene fusion APL. Through comparative analysis, this variant APL demonstrates smaller and more densely packed azurophilic granules within the cytoplasm, finer nuclear chromatin, and an absence of Auer rods compared to typical APL. Additionally, we observed that the immunophenotype of these cells expresses CD34

Table 1. Characteristics of NUP98::RARA positive cases

Age	Gender	WBC ($\times10^9$/L)	Blast (%)	Hb (g/dL)	PLT ($\times10^9$/L)	DIC	Karyotype	ATRA/ATO/ Combination therapy	Chemotherapy	Outcome (OS)	Ref.
31	M	2.4	9.8	124	123	-	normal	ND	S	44 mo, alive	(6)
70	M	122.92	0	101	20	+	CK	ND	ND	dead	Our study

ND, no data; S, sensitive; mo, months; OS, overall survival; CK, complex karyotypes; DIC, disseminated intravascular coagulation.

compared to typical APL, indicating a more primitive nature of these promyelocytes [12, 13]. Based on these findings, we favor a diagnosis of variant microgranular M3 (M3v). The findings have deepened our understanding of this APL subtype.

The co-occurrence of NUP98::RARA fusion and isochromosome i(17)(q10) in APL remains exceptionally rare, with no established therapeutic protocols currently available for this variant subtype. Establishing effective therapeutic strategies for this genetically distinct APL entity constitutes a critical clinical imperative. Furthermore, the NUP98 rearrangement drives leukemogenesis through induction of genomic instability, promoting accumulation of secondary genetic alterations that collectively contribute to its characteristically dismal prognosis [14-17]. The NUP98::RARA fusion transcript contains domains of GLEBS and GLFGs that can activate transcription and the main functional domains of RARA (DBD, LBD) (**Figure 2D**), which indicates that it may have the same pathogenesis and characteristics as those of PML::RARA. Chromosome i(17)(q10) is an unbalanced chromosome and associated with the duplication and deficiency of nucleic acid involved in the development and progression of hematologic malignancies [18]. It is quite rare and accounts for approximately 0.6-4.9% in APL cases [19]. However, there is no effective or specific treatment for these patients. APL patients with i(17)(q10) have been previously treated with ATRA, ATO and/or chemotherapy in the presence of PML::RARA, which results in a complete response. Previous studies showed that i(17)(q10) do not negatively affect the prognosis of patients treated with ATRA or chemotherapy, indicating that i(17)(q10) abnormality may not affect treatment pathways [20]. However, other authors have concluded that APL patients with i(17)(q10) have poor prognosis and short survival [21, 22]. Successful APL treatment requires rigorous management of severe complications during induction therapy to prevent early death while established risk factors - including age, WBC/PLT levels, surface antigen profiles, and complex karyotypes (CK) - directly impact prognosis and survival [23-25]. Therefore, we compared and summarized these two cases (**Table 1**). The previous study showed that a patient with the NUP98::RARA is sensitive to 3+7 chemotherapy and have long survival times. But that patient was 31 years old with low WBC (2.4×10^9/L), high PLT (123×10^9/L) and normal karyotypes, which classified as low risk [6, 26]. Comparing with this case, our patient was 70 years old with high WBC (122.92×10^9/L), low PLT (20×10^9/L) and CK. These are the high-risk factors affecting the prognosis and contributing to the early death of this patient [25, 27-29]. In addition, our case has two transcripts and is likely to have two cloned APL cells. Although one of the transcripts was identical to the one previously found, it could only suggest that its pathogenesis was the same. But the other transcript is more complex, with different break points, a longer sequence, and non-coding sequences inserted. This could lead to more unpredictable outcomes.

Current evidence regarding therapeutic efficacy, prognostic outcomes, and survival patterns in this APL variant remains critically deficient. The clinical utility of ATRA and arsenic trioxide regimens requires validation through prospective multicenter trials, contingent upon sufficient case accrual. Systematic molecular profiling may identify novel therapeutic vulnerabilities in this genetically distinct APL subtype.

Conclusions

We reported the first case of variant APL with two transcripts of NUP98::RARA and i(17)(q10) abnormality. These results indicate that the fusion gene is a reproducible abnormality. Additionally, the findings suggest that the fusion gene can have multiple subtypes. This patient received treatment with hydroxycarbamide, ATRA, RIF, and HTT, but unfortunately died due to cerebral hemorrhage with

cerebral infarction few days later. It is anticipated that patients with NUP98::RARA and i(17)(q10) may have an unfavorable response to ATRA combined with ATO/RIF therapy and a poor prognosis leading to short survival.

Disclosure of conflict of interest

None.

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