Identification of a novel *NUP98-RARA* fusion transcript as the 14th variant of acute promyelocytic leukemia

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To the Editor:

Until now, thirteen *RARA* fusion variants, including *ZBTB16, NPM1, NUMA1, STAT5B, PRKAR1A, FIP1L1, BCOR, NABP1, TBLR1, GTF2I, IRF2BP2, FNDC3B* and *STAT3*, have been reported in acute promyelocytic leukemia (APL).[1] Here, we report another novel *NUP98-RARA* fusion in an APL patient lacking the t(15;17)(q22;q12)/*PML-RARA* fusion, which represents the 14th variant of APL.

A 31-year-old man was admitted due to having a fever and feeling weak, and his blood count showed a white blood cell count of 2.4×10^9/L, including 29.5% promyelocytes, a hemoglobin level of 124 g/L, and a platelet count of 123×10^9/L. His prothrombin time, fibrinogen, fibrin degradation products and D-dimer levels were 16.3 s (ref.: 9.4-12.5 s), 85 mg/dl (ref.: 200-400 mg/dl), 82.4 μg/ml (ref.: 0-5 μg/ml) and 19690 ng/ml (ref.: 0-243 ng/ml), respectively. His activated partial thromboplastin time was within the normal range.

A bone marrow (BM) smear showed hypercellularity, with 78.4% hypergranular promyelocytes and 9.8% blasts (Figure 1A, left). The BM histochemistry showed a typical pattern for APL (Figure 1A, right). The leukemia cells expressed myeloperoxidase, CD117, CD123, CD33, CD13, CD9, CD64, and HLA-DR and partially expressed CD34; however, these cells lacked

the expression of CD11b, CD38, CD14, CD15, CD16, CD19, cCD79a, CD7, cCD3, and TdT. The presumptive diagnosis of this patient was the microgranular variant of APL. There was few Auer Rod and it is occasionally seen in a few APL cells. However, both the reverse transcription-polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH) failed to detect the *PML-RARA* transcript in the BM. The karyotype analysis revealed 46, XY.

On the first day after having a suspected diagnosis of APL, which was based on the morphological and immunophenotypical findings, the patient started an all-trans retinoic acid (ATRA) treatment. The white blood cell counts increased from 2.4×10^9/L to 12.2×10^9/L after 9 days. Because there was no evidence of a PML-RARA rearrangement with t(15;17), ATRA was stopped. The patient started induction chemotherapy composed of idarubicin and cytarabine on the 10th day. The patient achieved complete remission (CR) after one cycle of induction chemotherapy. He then received consolidation therapy, which included a high-dose of cytarabine for 3 cycles and “3+7”-based regimens for 3 cycles. To date, he has maintained a CR status for 44 months.

To search for a potential fusion gene, we used the patient’s BM samples collected at diagnosis to perform RNA sequencing using HiSeq (llumina, Inc.,

San Diego, CA, USA). After analyzing the data by STAR-Fusion and adjusting using FusionInspector software, the patient was shown to have a novel fusion transcript within chromosome 11, in which NUP98 was fused to *RARA*. To confirm this fusion, the following pair of primers was designed to perform PCR using cDNA: forward (at *NUP98* exon 2): 5'-ggatttaatactacgacagccactttg-3' and reverse (at *RARA* exon 3): 5'-ctctaccccgcatctacaagc-3'. An expected band of approximately 136 bp was visualized by gel electrophoresis, and the PCR product was analyzed by Sanger sequencing. The expected protein sequences were translated from the *NUP98-RARA* transcript (Figure 1B).

Self-binding to form homodimers and binding to RXR to form heterodimers are key features of many leukemia fusion proteins in mediating transcriptional deregulation and leukemogenesis.[2] To examine whether NUP98-RARA can form homodimers and heterodimers, coimmunoprecipitation (Co-IP) assays were performed with differentially tagged proteins in human 293T cells. Similar to other RARA fusion proteins, Myc-tagged NUP98-RARA could bind with His-tagged NUP98-RARA (Figure 1C), which indicated that NUP98-RARA could self-associate and form homodimers. In addition, NUP98-RARA could interact with RXR and form heterodimers (Figure 1D). To further assess the impact of retinoid/rexinoid on the homodimer formation and NUP98-RARA/RXR

interaction, Co-IP assay was performed by using these protein complexes in the absence or presence of 1 μM ATRA. Both the interactions of NUP98-RARA/NUP98-RARA and NUP98-RARA/RXR could be suppressed by treating with ATRA (Figure 1E and 1F).

To further investigate the cellular localization of the NUP98-RARA fusion protein, His-tagged versions of these proteins were expressed in 293T cells (Figure 1G). Using immunofluorescence and immunoblotting, we found that NUP98-RARA was predominantly located in the nucleus, and a small amount of it was in the cytoplasm (Figure 1H). NUP98-RARA fusion proteins have acquired an intracellular localization pattern that is distinct from that of the fusion partners (NUP98 and RARA). To further determine the domain within RARA needed for mediating the aberrant localization, we selectively deleted the DBD or LBD of RARA to obtain mutants of NUP98-RARA (ΔDBD) and NUP98-RARA (ΔLBD). While NUP98-RARA (ΔLBD) had a similar cellular distribution as the wild-type NUP98-RARA protein (Figure 1H), NUP98-RARA (ΔDBD) exhibited a decreased distribution of intranuclear proteins (Figure 1H), indicating a critical function of the DBD in determining the subcellular localization of the NUP98-RARA fusion protein.

We next found that NUP98-RARA was downregulated both in the nucleus and cytoplasm by treating with ATRA (1 μM) but not by treating with daunorubicin (100 nM) (Figure 1I). Surprisingly, combined treatment of ATRA (1 μM) with daunorubicin (100 nM) almost diminished the expression of NUP98-RARA (Figure 1I). More specifically, the expression of the NUP98-RARA fusion protein began to decrease significantly after 8 hours of treatment with 1 μM ATRA but showed no difference in the expression after 12 hours of treatment with 100 nM daunorubicin (Figure 1J). Consistent with the observation of the downregulated expression in nucleus and cytoplasm, after 2 hours of the combined treatment of ATRA and daunorubicin significantly reduced the total protein level of NUP98-RARA (Figure 1J). Furthermore, non-APL U937 leukemia cells stably transfected with *NUP98-RARA* were employed to detect the potential efficacy of ATRA and/or daunorubicin treatments. *NUP98-RARA*-transformed U937 cells treated with 1 μM ATRA alone underwent differentiation-associated changes with lobulation of nuclei, and an increase in CD11b expression concurred with the morphological changes (Figure 1K and 1L). Furthermore, coadministration of ATRA and daunorubicin induced typical apoptotic morphological changes (e.g. nuclear condensation) (Figure 1K). While wild-type U937 cells showed a negligible differentiation ratio after ATRA

treatment at a concentration of 1 μM, *NUP98-RARA*-transformed U937 cells were extremely sensitive to the coadministration of ATRA and daunorubicin (Figure 1L). These data revealed that the combined therapy of ATRA and daunorubicin may have potential therapeutic effects for leukemia patients with *NUP98-RARA*.

The NUP98 gene encodes a protein component of the nuclear pore complex (NPC) that regulates the nucleocytoplasmic transport of proteins and mRNAs.[2,3] NUP98 contains a domain with a GLFG repeat that can activate transcription. Chimeric transcripts formed by the NUP98 N-terminal GLFG repeats fused to the C-terminus of partner proteins are expressed in all reported NUP98 fusions, suggesting that the NUP98 N-terminus may be important for leukemogenesis.[3] Here, we report the first case of RARA as a new NUP98 partner that has a similar cellular localization to other NUP98-fusion proteins.[2] Moreover, we demonstrated that NUP98-RARA could self-associate and form homodimers and was able to coimmunoprecipitate with RXR and form heterodimers, which may suppress the transcription of downstream genes.

How to treat rare variants of APL is an important issue.[4,5,6] Although we demonstrated that NUP98-RARA showed sensitivity to ATRA ex vivo, we could not assess this treatment in this patient because of early discontinuation of

ATRA. Compared with PML-RARA, NUP98-RARA also harbors the main functional domains of DBD and LBD in the fusion protein, which partially explains their similar biological properties. Combination of daunorubicin and ATRA obviously decreased NUP98-RARA expression and showed sensitivity to U937 cells expressing NUP98-RARA, which may partially explain the favorable outcome of this patient who received an anthracycline-based chemotherapy treatment.

In conclusion, we describe here a novel *NUP98-RARA* gene rearrangement as the 14th variant of APL, which exhibits morphological and immunophenotypical features of APL. The favorable outcome of this patient with a standard 3+7 chemotherapy did not allow us to ascertain the sensitivity of NUP98-RARA to ATRA due to the early discontinuation of the drug. Further studies are needed to better understand the biological properties, especially its sensitivity to ATRA, of this new NUP98-RARA fusion protein.

AUTHOR CONTRIBUTION

H.H.Z., K.L., and S.Z.Z. designed the research and analyzed data. M.C.Y., F.W., J.J., and Y.J.L. performed research. H.H.Z. and K.L. wrote the paper.

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Reference

1. Yao L, Wen L, Wang N, et al. Identification of novel recurrent STAT3-RARA fusions in acute promyelocytic leukemia lacking t(15;17)(q22;q12)/PML-RARA. Blood. 2018;131(8):935-939.
2. Qiu JJ, Zeisig BB, Li S, et al. Critical role of retinoid/rexinoid signaling in mediating transformation and therapeutic response of NUP98-RARG leukemia. Leukemia. 2015;29(5):1153-62.
3. Such E, Cervera J, Valencia A, et al, A novel NUP98/RARG gene fusion in acute myeloid leukemia resembling acute promyelocytic leukemia. Blood. 2011;117(1):242-5.
4. Dowse RT, Ireland RM. Variant ZBTB16-RARA translocation: morphological changes predict cytogenetic variants of APL. Blood. 2017;129(14):2038.
5. Qin YZ, Huang XJ, Zhu HH. Identification of a novel CPSF6-RARG fusion transcript in acute myeloid leukemia resembling acute promyelocytic leukemia. Leukemia. 2018 ;32(10):2285-2287.

6. Liu T, Wen L, Yuan H, et al. Identification of novel recurrent CPSF6-RARG fusions in acute myeloid leukemia resembling acute promyelocytic leukemia. Blood. 2018 ;131(16):1870-1873.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Figure legends

Figure 1. Morphology of leukemic/patient's myeloid blasts, schematic representation, subcellular localization pattern and drug sensitivity of NUP98-RARA fusion proteins. (A, Top) Several promyelocytes with a hypergranulated cytoplasm and invaginated nuclei are shown. (Wright-Giemsa stained bone marrow smear, × 1000). (A, Bottom) The peroxidase stain shows strong positivity. (B) Expected protein sequences translated from the NUP98-RARA transcript. (C-F) Identification of homodimerization and heterodimerization of NUP98-RARA fusion proteins by Co-IP. Cell lysates from cotransfected 293T cells were immunoprecipitated with either an anti-His or anti-Myc antibody and western blotted with an anti-RXR or anti-His antibody. Input fractions were blotted with the indicated antibodies. (C) Co-IP between His-tagged NUP98-RARA and Myc-tagged NUP98-RARA. (D) Co-IP between His-tagged NUP98-RARA and RXR. (E and F) Co-IP between His-tagged NUP98-RARA and RXR or Myc-tagged NUP98-RARA in the absence or presence of 1 μM ATRA for 12 hours. (G) Schematic representation of the following His-tagged proteins: NUP98-RARA, NUP98, RARA, Δ-DBD and Δ-LBD mutants. (H) The expression of His-tagged proteins in 293T cells was analyzed by fluorescence microscopy. DNA was visualized by counterstaining

with DAPI (blue). (I) The effects of ATRA and/or daunorubicin on the expression of NUP98-RARA protein in cytoplasmic (C) and nuclear (N) fractions of 293T cells transfected with NUP98-RARA plasmids. Lamin A/C and actin were used as nuclear and cytoplasmic markers, respectively. (J) The effects of ATRA and/or daunorubicin on the expression of the NUP98-RARA protein in the total fractions of 293T cells transfected with NUP98-RARA plasmids for the indicated times. (K) Morphological changes in NUP98-RARA transformed cells treated with ATRA and daunorubicin, alone or in combination. After the treatment with 1 μM ATRA and 0.1 μM daunorubicin, alone or in combination, for 12 h, morphological changes in U937 cells stably expressing NUP98-RARA (U937-NUP98-RARA) were evaluated by Wright-Giemsa staining. (L) The effects of ATRA and/or daunorubicin on the cellular differentiation of NUP98-RARA transformed cells. Wild-type U937 cells and U937-NUP98-RARA cells were treated with ATRA and daunorubicin alone or in combination for 12 h. The expression level of CD11b in these cells was evaluated by flow cytometry. The data are presented as the means ± s.e.m. of three independent assays. An unpaired two-sided Student's t-test was used to compare two groups. *$p < 0.05$, ***$p < 0.001$.