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Letter to the Editor

A novel entity of acute myeloid leukaemia with recurrent RARG-rearrangement resembling acute promyelocytic leukaemia

Recently, an increasing number ofacute myeloid leukaemia (AML) patients with retinoic acid receptor gamma (*RARG*)-rearrangement were identified using next generation sequencing (NGS). Until now, *NUP98-RARG, PML-RARG* and *CPSF6-RARG*rearrangements have been reported [1–6]. All of these patients show classic features of acute promyelocytic leukaemia (APL) including their clinical presentation, coagulation abnormality, and morphological and immunophenotypingfeatures of the bone marrow. However, the lack of retinoic acid receptor alpha (*RARA*)-rearrangement inthese types of patients has renderedtheir classification and treatment options difficult. Therefore, an urgent need exists to study the classification, underlying molecular pathology and therapeutic approaches of AML patients with *RARG*-rearrangement.

In this study, we first report the clinical and experimental results of one case of AML with *NUP98-RARG* rearrangement and review our current understanding of AML patients with *RARG*-rearrangement.

On April 17, 2018, a 45-year-old woman was admitted to our hospital due to weakness and skin ecchymosis. Her blood count showed a white blood cell count of 0.2×10^9/L, including 27% promyelocytes, a haemoglobin level of 66 g/L and a platelet count of 60×10^9/L. Her prothrombin time, activated partial thromboplastin time, and fibrinogen levels were 12.3 s (ref. 10–14 s), 25.4 s (ref. 20–40 s), and 129 mg/dl (ref. 200–400), respectively. A bone marrow (BM) smear showed hypercellularity, with 94.5% hypergranular promyelocytes with (Fig. 1, A). The BM histochemistry showed a staining pattern of typical APL (Fig. 1B). The leukaemic cells expressed myeloperoxidase (MPO),CD117, CD33, CD13, CD38 and CD64 but lacked the expression of CD34, CD11b, HLA-DR, CD56,CD14 and other T- or B-lymphoid-related markers. The presumptive diagnosis of this patient was APL. However, reverse transcription polymerase chain reaction (RT-PCR) toamplify the *PML-RARA* fusion transcript and conventional karyotyping tosearch for the t(15;17) translocation were also negative.-Considering thepresumed diagnosis of APLbased on the morphological and immunophenotypical findings, we started treatment with all-trans retinoic acid (ATRA) 40 mg per day and arsenic trioxide (ATO) 10 mg per day on day one. After treatment for 21 days, the coagulation abnormality did not improve and differentiation syndromes did not occur. The white blood cell count was 0.26×10^9/L, including40% promyelocytes, a haemoglobin level of 74 g/L and a platelet count of 26×10^9/L. The percentages of promyelocytes, myelocytes, and metamyelocytes in the bone marrow were 90%, 0.5% and 0%, respectively, after treatment for 21 days. Because the patient showed compete resistance to ATRA and ATO, the treatment wasswitched to cytarabine 100 mg per day and aclarubicin 20 mg per day for seven daysas induction therapy. The patient died of severe blood infection with *Escherichia coli* 6 days after completing chemotherapy, which was 35 days after admission.

G-banding karyotype analysis of BM cells revealed a clonal translocation t(11;12)(p15;q13) in 16 of 20 metaphases analyzed (Fig. 1C). The *NUP98-RARG* mRNA was reverse-transcribed into cDNA using random hexamers, and PCR was performed using the following primers: *NUP98* exon 10 *NUP98F*: 5′-GGG CTT GGT GCA GGA TTT GG-3′, and *RARG* exon 7 *RARGR*: 5′-TGG GTC CGG TTC AGG GTC AGC-3′, which is the same as described elsewhere [1]. To confirm a NUP98-RARG fusion, an 881-bp product was specifically amplified from the patient's cDNA but not from the control. The transcript fusion product was co-amplified in the same reaction and sequenced. Sequence analysis of the *NUP98-RARG* fusion transcript revealed that *NUP98* exon 12 was fused in-frame to *RARG* exon 4 (Fig. 1D).The Wilms' tumour (WT1)-R445 W mutation was identified in addition to the *NUP98-RARG* fusion by a customized target next-generation sequencing that included 34 AML-related genes.

The BM mononuclear lysates from the patient were analyzed by Western blot with a rabbit anti-RARG antibody (Abcam, USA), which is as our previous report. 3 As shown in Fig. 1E, in addition to the wild type band (58 kD), a larger band (120 kD) corresponding to the NUP98 fused RARG was seen. The results of Western blot analysis demonstrated the NUP98-RARG fusion gene can lead to the formation of NUP98-RARG fusion protein, which support the diagnosis of AML with NUP98-RARG rearrangement. We further examined the cellular localization of the NUP98-RARG fusion protein, immunostaining of primary leukemic cells of the patient was used. As shown in Fig. 1F, NUP98-RARG fusion protein had a typically diffused intranuclear pattern, which is consistent with the previous report [7].

A review of published cases of *RARG*-rearrangement AML help clarify the characteristics of the emerging subtypes of AML. Until now, seven patients have been reported including this case [1–6]. There are 3 males and 4 females with a median age of 45 years old. The median WBC and PLT were 1.68×10^9/L and 60×10^9/L, respectively. All of the patients had severe coagulation abnormality, classic M3 morphology with hypergranular promyelocytes, and a $CD34^-HLA^-DR^-CD11b^-CD13^+CD33^+CD117^+$ immunophenotype. AML patients with RARG-rearrangement showed resistance to ATRA and arsenic.

NUP98-RARG fusion derived from t(11;12)(p15;q13) with APL-like morphologic and immunophenotypic features was first reported by Such and colleagues in 2011 [1]. Since ATRA was given simultaneously with chemotherapy to the patient, the authors were unable to evaluate the effect of ATRA *in vivo*. Three years later, the *in vitro* studies by the same group using relapsed primary blasts of the same patient showed that AML with a *NUP98-RARG* rearrangement is resistant to ATRA [6]. However, Qiu and colleagues demonstrated a high sensitivity to ATRA in inducing differentiation and apoptosis of *NUP98-RARG* transformed HeLa cells [7]. For the first time, our case provides strong evidence of both ATRA and ATO resistance to NUP98-RARG blasts in the clinic, though the underlying mechanism is largely unknown. The apparent paradox of ATRA resistance in vivo and responsiveness in vitro may be

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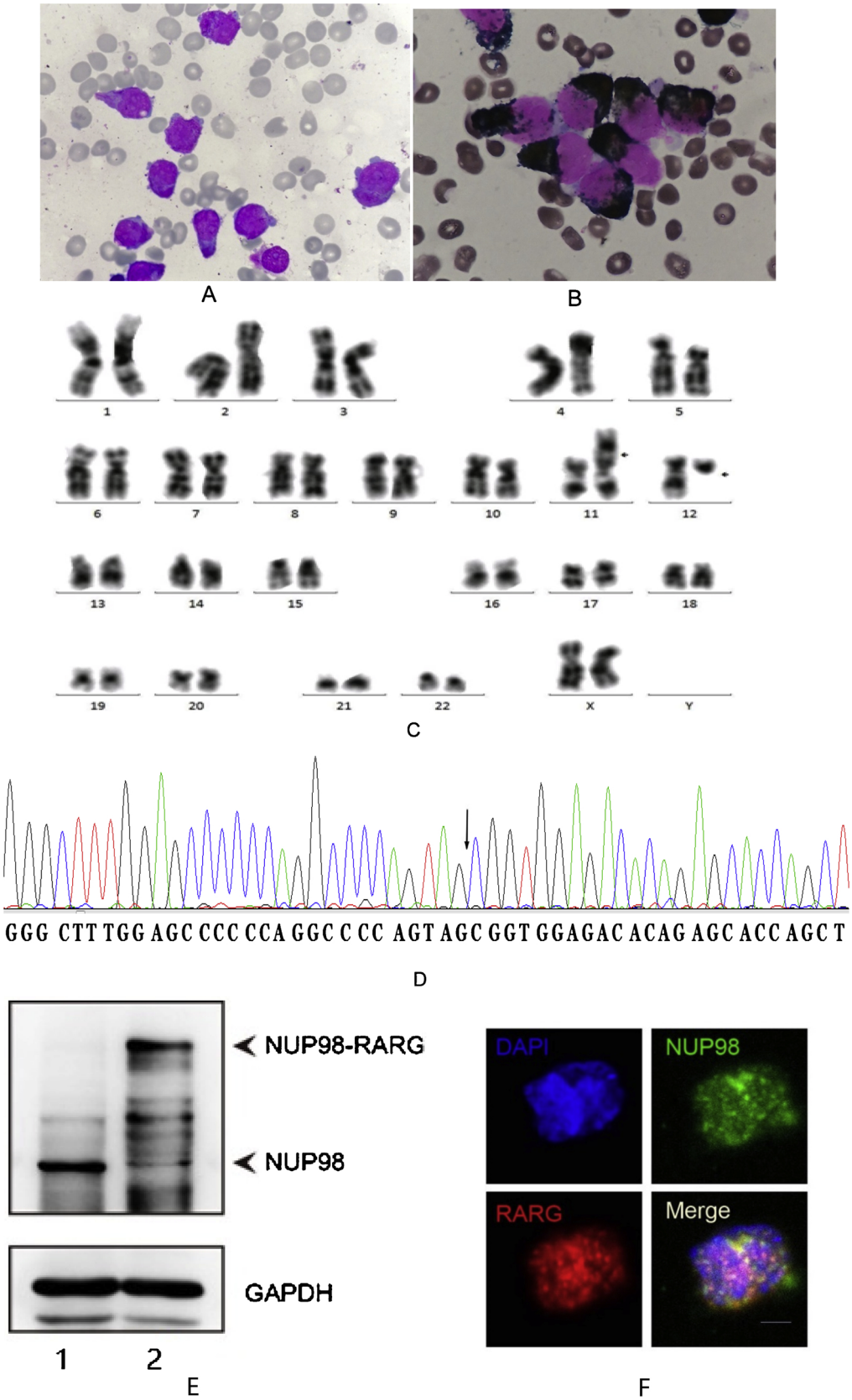

Fig. 1. Morphology, karyotype and molecular analysis of the *NUP98-RARG* fusion. (A) Several promyelocytes, with hypergranulated cytoplasm, invaginated nuclei and Auer rods are shown. (Wright-Giemsa stained bone marrow smear, × 1000). (B) The peroxidase stained bone marrow smear showed strong positivity. (C) G-banded karyotype showing 46 XX, t (11;12)(p15;q13) [6]/46 XX [4].(D) Nucleotide sequences surrounding the NUP98-RARG fusion region. (E) Expression of NUP98 and NUP98-RARG was detected by western blotting of leukaemia cell proteins derived from the NUP98-RARG patient (Line 2) compared with an APL patient (Line 1). (F) Co-localization of NUP98 (green) and RARG (red) was detected by immunostaining of primary human leukaemia cells derived from the NUP98-RARG patient (blue, DAPI). Data are representative images from three assays. Scale bar, 2 μm (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

due to the use of a relatively short-term in vitro culture assay or/and their acquisition of additional mutations that conferred the resistance [6,7]. The acquisition of additional mutations such as WT1 mutations (R445 W in our case, R462Q, E340 and S1381in CPSF6-RARG patients) might confer the resistance to ATRA in patients with RARG-rearrangement [3,5], a finding that needs to be validated further.

From the review of the literature, emerging evidencesuggests that AML with *RARG*-rearrangement is a novel entity of AML because

itharbours unique clinical, coagulation, morphology and immunophenotyping features typically seen in APL. Most importantly, ATRA and ATO-resistance and high early death rate following conventional chemotherapy indicate the poor outcome of this entity compared toclassic APL and other AM.L. Some basic studies have provided preliminary evidence to elucidate the differences between RARG- and RARA-rearrangement [7–9].

Future studies are needed to define the underlying mechanisms contributing to leukaemogenesis, and new agents capable of disrupting the RARG-related signalling pathway may represent a potential therapeutic target for AML with *RARG*-rearrangement. RXR agonist treatment of cells transformed by NUP98-RARG RXR-interaction defective mutant resulted in significantly reduced colony numbers [7], suggesting that RXR agonists has potential therapeutic application in NUP98-RARG cells. The optimal treatment regimen awaits to be established in the future. In summary, the similarity of the clinical characteristics and unfavourable outcome may support the notion of a novel entity of AML with an*RARG*-rearrangement.

Statement of conflict of interest

The authors declare no conflicts of interest.

Authorship

Contribution: H.-H.Z.,H.-Y.Y. and H.L. had full access to all of the data and takeresponsibility for the data integrity and analysis; X.-H.C. and

Y.-C.L. contributed to molecular analysis; Y.S. and L.-F.L.performed cytogeneticanalysis; H.L. and S.Z.provide the clinical data, and all authors contributed to the final draft.

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