A novel \textbf{NPM1-RARG-NPM1} chimeric fusion in acute myeloid leukaemia resembling acute promyelocytic leukaemia but resistant to all-trans retinoic acid and arsenic trioxide

Xue Chen\textsuperscript{1}, Fang Wang\textsuperscript{1}, Yang Zhang\textsuperscript{1}, Wen Teng\textsuperscript{1}, Panxiang Cao\textsuperscript{1}, Xiaoli Ma\textsuperscript{1}, Mingyue Liu\textsuperscript{1}, Yaoyao Tian\textsuperscript{2}, Tong Wang\textsuperscript{1}, Daijing Nie\textsuperscript{1}, Jing Zhang\textsuperscript{1}, Hongxing Liu\textsuperscript{1,3,4} and Wei Wang\textsuperscript{2}

The \textit{RARG} gene is a member of the nuclear hormone receptor superfamily and shares high homology with \textit{RAR}. \textit{RAR} is involved in translocation with \textit{PML} in acute promyelocytic leukaemia (APL). Little is known about \textit{RARB} or \textit{RARG} rearrangement. \textit{RARG} fusions were reported in only five APL patients and the partner genes were \textit{NUP98}, \textit{PML} and \textit{CPSF6}. Here, we report \textit{NPM1} as a new partner gene of \textit{RARG} and identify a unique \textit{NPM1-RARG-NPM1} chimeric fusion for the first time in an old male with morphological and immunophenotypical features of hypergranular APL but lacking response to all-trans retinoic acid (ATRA) and arsenic trioxide (As\textsubscript{2}O\textsubscript{3}) therapy. The structural features of the fusion transcript may account for the clinical resistance of the patient. \textit{RARG} fusion is rare but recurrent in APL, further investigation in larger cohorts is expected to account for frequency, clinical characteristics and outcomes of \textit{RARG}-translocation in APL.

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BACKGROUND

Acute promyelocytic leukaemia (APL) is characterised by the \textit{PML-RARA} fusion caused by t(15;17)(q22;q12) translocation. Rarely, APL cases carry gene fusions involving \textit{RARG}, which is a member of the same retinoid acid receptor (RAR) family and shares high homology (90\%) with \textit{RAR} and \textit{RARB}. \textit{RARA} is involved in translocation with \textit{PML} in acute promyelocytic leukaemia (APL). Little is known about \textit{RARB} or \textit{RARG} rearrangement. \textit{RARG} fusions were reported in only five APL patients and the partner genes were \textit{NUP98}, \textit{PML} and \textit{CPSF6}. Here, we describe the first case with a novel \textit{NPM1-RARG-NPM1} chimeric fusion in an old male with morphological and immunophenotypical features of hypergranular APL but lacking response to all-trans retinoic acid (ATRA) and arsenic trioxide (As\textsubscript{2}O\textsubscript{3}) therapy.

METHODS

Case reports

A 69-year-old man was admitted because of 2-week history of asthenia and dizziness. Blood tests showed haemoglobin level of 123 g/L, platelet count of 204 × 10\textsuperscript{11}/L, and white blood cell count of 1.5 × 10\textsuperscript{9}/L. Fibrinogen, fibrin degradation products and D-dimer levels were 1.72 g/L (reference, 2.00–4.00 g/L), 20 μg/ml (reference, 0–5.0 μg/ml) and 5.25 μg/ml (reference, 0–0.23 μg/ml). Prothrombin time and activated partial thromboplastin time were 12.4 s (reference, 8.9–13.3 s) and 32.2 s (reference, 25.0–45.0 s), respectively.

Morphologic examination of bone marrow (BM) smears disclosed infiltration by 56\% of hypergranular promyelocytes (Fig. S1a). These cells demonstrated strong and diffuse reactivity to myeloperoxidase cytochemical staining, which often covered the nucleus and consistent with the characteristics of APL (Fig. S1b). The blasts were positive for CD13, CD33, CD45, CD9, CD64 and cytoplasmic myeloperoxidase, partially positive for HLA-DR, CD117, CD56 and CD123, but negative for CD34, CD14, CD38, CD11b, CD16 and other T- or B-lymphoid related markers. The chromosome karyotype was normal and t(15;17)(q22;q12) translocation was not detected by karyotyping (Fig. S1c). Multiplex-nested reverse transcription polymerase chain reaction (RT-PCR) designed to amplify 36 fusion transcripts, including \textit{PML-RARA}, \textit{ZBTB16-RARA} and \textit{NPM1-RARA} showed 3 abnormal positive bands in one reaction which was designed to amplify \textit{NPM1-RARA}. Sanger sequencing of PCR products revealed \textit{NPM1-RARG} fusions of \textit{NPM1} exon 4 to \textit{RARG} partial exon 1, exon 2 or exon 4, respectively (Fig. S1d). The extensive homology between \textit{RARA} and \textit{RARG} made it possible to amplify \textit{NPM1-RARG} using primers designed to amplify \textit{NPM1-RARA}.

The patient was treated with As\textsubscript{2}O\textsubscript{3} (10 mg/d, days 1–34) and showed no response. Then he was switched to ATRA therapy (50 mg/d, days 35–70) after the confirmation of \textit{NPM1-RARG} rearrangement. The \textit{NPM1-RARG} transscripts remained positive and were highly expressed in both peripheral blood and BM samples of the patient during the course of treatment. He refused to receive chemotherapy and died 8 months after diagnosis.
Whole genome sequencing
To clarify the genomic breakpoints in \textit{NPM1} and \textit{RARG}, 30x whole genome sequencing (WGS) was performed on genomic DNA of BM sample using HiSeq X Ten (Illumina, Inc., San Diego, CA) after approval by the ethics committee at the 2nd Afiliated Hospital of Harbin Medical University. Raw reads in fastq were pre-processed and controlled for quality using fastp, followed by rapid genome analysis using speedseq with default parameters. Structural variants were called from speedseq with default options and the next annotation tool was AnnotSV.

Targeted next-generation sequencing and mutation analysis
Mutational hotspots or whole coding regions of 86 genes that are known to be frequently mutated in haematologic malignancies were sequenced using a targeted, multiplexed amplicon-based high-throughput sequencing protocol as we previously reported.

RESULTS
Laboratory, morphology and immunophenotypic analysis of the patient suggested diagnosis of hypergranular APL. \textit{IDH1} R132H and \textit{SRSF2} P95_R102del mutations were identified.

WGS analysis revealed breakpoints in intron 4 of \textit{NPM1} and 5'-untranslated region (5'-UTR) of \textit{RARG}. Interestingly, two more breakpoints in \textit{NPM1} intron 10 and \textit{RARG} intron 9 were identified. Both \textit{NPM1} intron 4-\textit{RARG} 5'-UTR and \textit{RARG} intron 9-\textit{NPM1} intron 10 genomic fusions were confirmed by Sanger sequencing. Hence the genomic alterations of this patient were a deletion of 16,360 bp of \textit{NPM1} from intron 4 to intron 10 accompanied by an insertion.

Fig. 1 Identification of a novel \textit{NPM1-RARG-NPM1} chimeric fusion in a APL case lacking t(15;17)(q22;q12)/PML-RARA. \textbf{a} WGS found that \textit{NPM1} and \textit{RARG} each has two breakpoints (shown in red arrows). Sequencing chromatogram showed the two genomic junction sequences (\textit{NPM1} intron 4-\textit{RARG} 5'-UTR and \textit{RARG} intron 9- \textit{NPM1} intron 10). \textbf{b} RT-PCR and sequencing of the PCR products verified the presence of \textit{NPM1-RARG-NPM1} chimeric fusion with three kinds of fusion transcripts. \textbf{c} Expected protein sequences translated from the three fusion transcripts. The same oligomeric amino acid tail (VSLRK) as in C-terminal region of all mutant \textit{NPM1} that frequently occurred in AML was shown in yellow.
of 23,479 bp of RARG from 5′ UTR to intron 9 (Fig. 1a). Moreover, RT-PCR and Sanger sequencing verified the presence of three NPM1-RARG-NPM1 transcripts presumably derived from alternative splicing: NPM1 (exon 1–4) — RARG (partial exon 1-exon 9) — NPM1 (exon 11), NPM1 (exon 1–4) — RARG (exon 2–9) — NPM1 (exon 11) and NPM1 (exon 1–4) — RARG (exon 4–9) — NPM1 (exon 11) (Fig. 1b). The microdeletion of NPM1 and microinsertion of RARG at the genomic level were too subtle to be found by karyotype analysis.

The NPM1 5′-region encoding the nucleoplasm domain was fused to the DNA-binding domain (DBD) of RARG in all three transcripts. Deletion of RARG exon 10 led to 25 amino acids loss of the ligand-binding domain (LBD) of RARG. Notably, the three transcripts generated the same C-terminal oligomeric amino acid tail (VSLRK) as in all mutant NPM1 that frequently occurred in AML5 due to the 3′ end fusion of NPM1 exon 11 and frameshift coding. The two critical C-terminal tryptophan (W) residues at positions 288 and 290 which are necessary for nucleolar localisation of NPM1 were also altered6 (Fig. 1c).

**DISCUSSION**

The NPM1 gene encodes nucleophosmin, which is a highly conserved nucleo-cytoplasmic shuttling protein that shows restricted nucleolar localisation. Mutations or translocations involving NPM1 gene cause cytoplasmic ectopia of nucleophosmin and are associated with several haematological malignancies, especially the bio pathogenesis of AML.6 NPM1-RARA has been reported as a very rare variant of RARA translocations in APL (Fig. S2a). In this case, the NPM1-RARG-NPM1 fusion leads to both impairment of NPM1 protein and abnormal of RARG. The missing of NPM1 exon 5–9 and the mutation-like C-terminus of the NPM1-RARG-NPM1 transcripts may result in impaired function and ectopia of nucleophosmin in cytoplasm and contribute to the impaired differentiation and leukaogenesis.

RARG, RARA and RARB are nuclear hormone receptors functioning as ligand-dependent transcriptional activators that interact specifically to modulate transcription of DNA elements, and all have highly conserved DBD and LBD.8 Fusions and aberrations of RARs contributed to hematopoietic differentiation arrest at promyelocytes stage and constitute the basis for therapeutic response of ATRA-induced differentiation therapy. Although very rare, translocations involving RARB (TBL1XR1-RARB)9 and RARG (NUP98-RARG, PML-RARG, and CPSF6-RARG)10–14 have been reported in APL. As in PML-RARA and other RARA fusions, RARG and RARB rearrangements in reported cases preserve both DBD and LBD10–12,14 (Fig. S2b, e). In the present case, deletion of RARG exon 10 caused 25 amino acids loss of LBD thus may result in impaired ATRA binding affinity (Fig. S2f). On the other hand, the fusion partner of RARG is NPM1 rather than PML may make the patient resistant to As2O3 due to lack of As2O3 binding site.10 These are in line with the clinical resistance of ATRA and As2O3 of the patient.

**CONCLUSION**

We report NPM1 as a partner gene of RARG in a patient morphologically resembling APL but lacking response to ATRA and As2O3 therapy for the first time and identify a unique NPM1-RARG-NPM1 chimeric fusion. RARG fusion with different partners is rare but recurrent in APL. Further investigation in larger cohorts is expected to assess frequency, clinical characteristics and outcomes of RARG-translocation in APL.

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