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CASE REPORT

Characterization of a rarely reported *STAT5B/RARA* gene fusion in a young adult with newly diagnosed acute promyelocytic leukemia with resistance to ATRA therapy

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Abstract

The detection of *PML/RARA* or variant *RARA* rearrangements is critical for the diagnosis and treatment of patients with newly diagnosed acute promyelocytic leukemia (APL). While most cases of APL harboring the *PML/RARA* fusion respond to all-trans retinoic acid (ATRA), some variant *RARA* rearrangements are ATRA insensitive. Herein, we report a 27-year-old male with newly diagnosed, rapidly progressive APL and a rarely described *STAT5B/RARA* fusion with known resistance to ATRA therapy. While the *PML/RARA* dual-color dual-fusion fluorescence *in situ* hybridization (FISH) probe study was negative, the *RARA* break-apart probe study revealed an atypical *RARA* rearrangement in 95% of nuclei. A next generation sequencing assay, mate-pair sequencing, was subsequently performed to further characterize the *RARA* rearrangement and identified the *RARA* gene fusion partner *STAT5B*.

Keywords Acute promyelocytic leukemia (APL), *RARA*, *STAT5B*, Next generation sequencing (NGS), Mate-pair sequencing (MPseq).

Clinical history

A 27-year-old male without a past medical history presented to the emergency room with a week of migratory muscle aches and sweats along with a 2-month history of progressive fatigue. A complete blood count revealed anemia (hemoglobin, 5 g/dL; reference, 13.2–16.6 g/dL), thrombocytopenia (platelet count, 24 × 10^3/μL; reference, 135–317 × 10^3/μL), and leukocytosis (white blood cell (WBC) count, 52 × 10^3/μL; reference, 3.4–9.6 10^3/μL). A subsequent bone marrow evaluation demonstrated marrow replacement (nearly 100%) by predominantly intermediate to large mononuclear and rare bilobed myeloid cells, some with microgranules in the cytoplasm (Fig. 1A–D). Flow cytometric analysis of the bone marrow aspirate demonstrated atypical myeloid cells (95% of analyzed cells) expressing CD13, CD33 and CD64 (dim), including a subset (10–15%) of cells that also expressed CD117, CD34, CD2 and CD4. HLA-DR and CD11b were negative (Fig. 1E–I). Taken together, the morphologic and immunophenotypic findings were consistent with a diagnosis of acute myeloid leukemia (AML) with features suggestive of acute promyelocytic leukemia (APL). The patient was started on all-trans retinoic acid (ATRA) 8 h after presentation and hydroxyurea was started to lower his WBC count.

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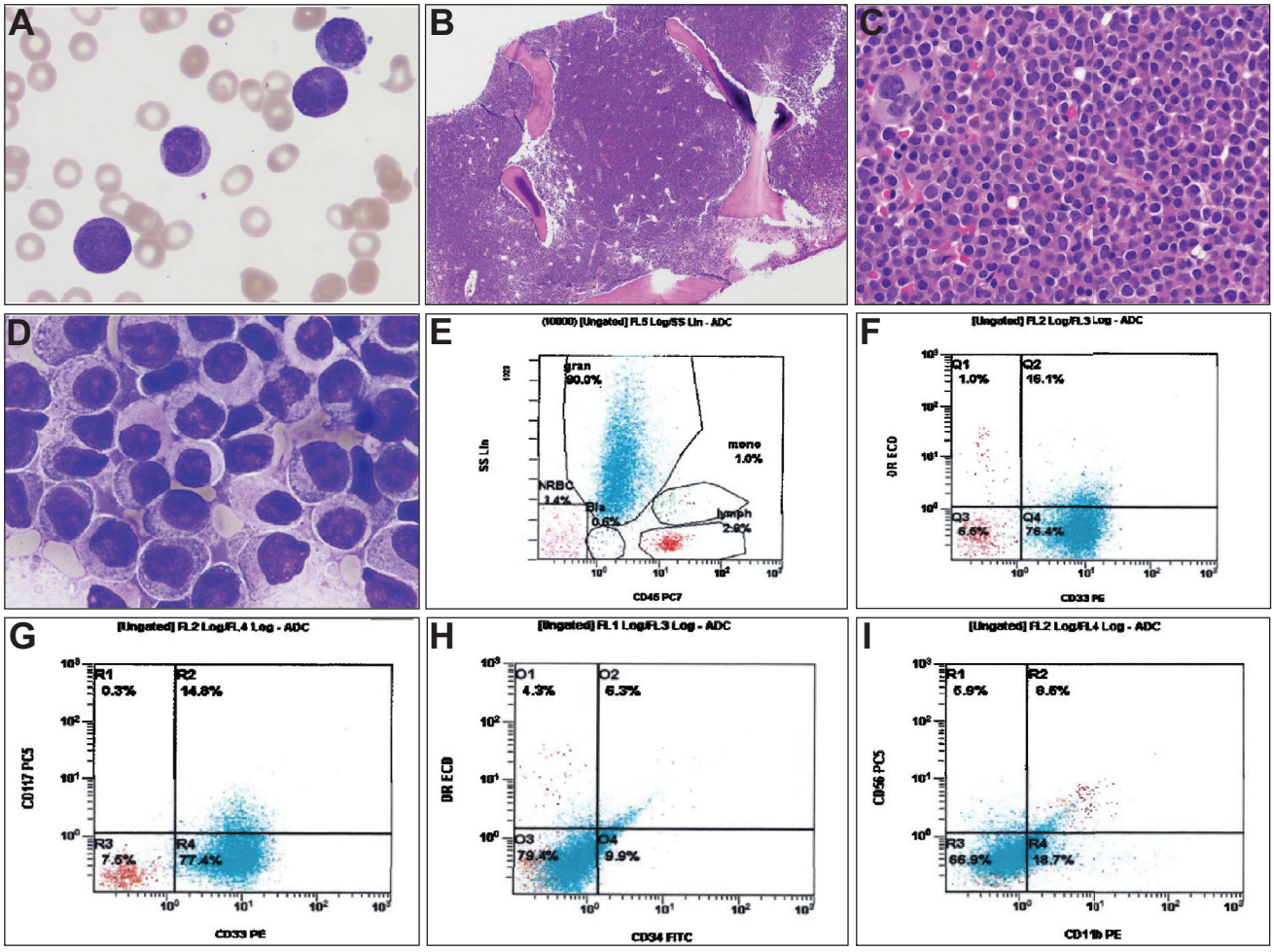

Fig. 1 Morphologic and immunophenotypic evaluation. (A**)** Peripheral blood smear showing many intermediate to large mononuclear and rare bilobed atypical myeloid cells, rare forms resembling promyelocytes with cytoplasmic granules (100 × oil objective). (B) Bone marrow core biopsy showing marked hypercellularity with nearly 100% replacement by atypical mononuclear and rare bilobed myeloid cells (10× objective). (C) Higher power view (40× objective) of the bone marrow core biopsy. (D) Bone marrow aspirate showing numerous mononuclear and rare bilobed atypical myeloid cells, some forms resembling promyelocytes with cytoplasmic granules. No Auer rods were identified (100 × oil objective). (E) Dot plot showing a large population of atypical myeloid cells in dimer CD45 and high side scatter region. (F–I) The atypical myeloid population is positive for CD33 and negative for HLA-DR, CD11b and CD56. A subset of these cells is positive for CD117.

Genomic analyses

Genomic studies were performed on the diagnostic bone marrow aspirate specimen. Conventional chromosome analysis revealed a paracentric inv(17)(q21q24) in 15 of 20 metaphases analyzed (Fig. 2A). Five metaphases were normal (46,XY). A comprehensive AML FISH panel, including the *PML/RARA* dual-color dual-fusion (D-FISH) probe set, was negative (Fig. 2B). Considering the morphologic and immunophenotypic features suggestive of APL and the abnormal chromosome 17 identified in the chromosome study, a *RARA* break-apart probe (BAP) was utilized to detect a potential variant *RARA* rearrangement. This probe set demonstrated loss of the 3′*RARA* BAP in 95% of 200 interphase nuclei (abnormal cutoff: ≥1.5%) (Fig. 2C). To further characterize this result, a next generation sequencing (NGS) strategy, mate-pair sequencing (MPseq), was performed. Consistent with the abnormal chromosome study, MPseq detected two separate paracentric 17q inversions involving a single chromosome 17. The first, inv(17)(q21.2q21.2), was detected with breakpoints located within the *RARA* gene (intron 2, NM_000964) and the *STAT5B* gene (intron 15, NM_012448) (Fig. 2D). The second, inv(17)(q21.2q24.3), was detected with breakpoints located within the proximal portion of exon 9 of the previously described *RARA* allele, and an intergenic region within 17q24.3. (Fig. 2E). These inversions are predicted to result in an in-frame 5′*STAT5B* (exons 1–15)/3′*RARA* (exon 3-proximal portion of exon 9) gene fusion, in addition to loss of the distal portion of exon 9 of the *RARA* allele involved in the second inversion (Fig. 2F).

Discussion

The accurate detection of *RARA* gene fusion partners in APL is critical as some *RARA* gene fusions fail to respond

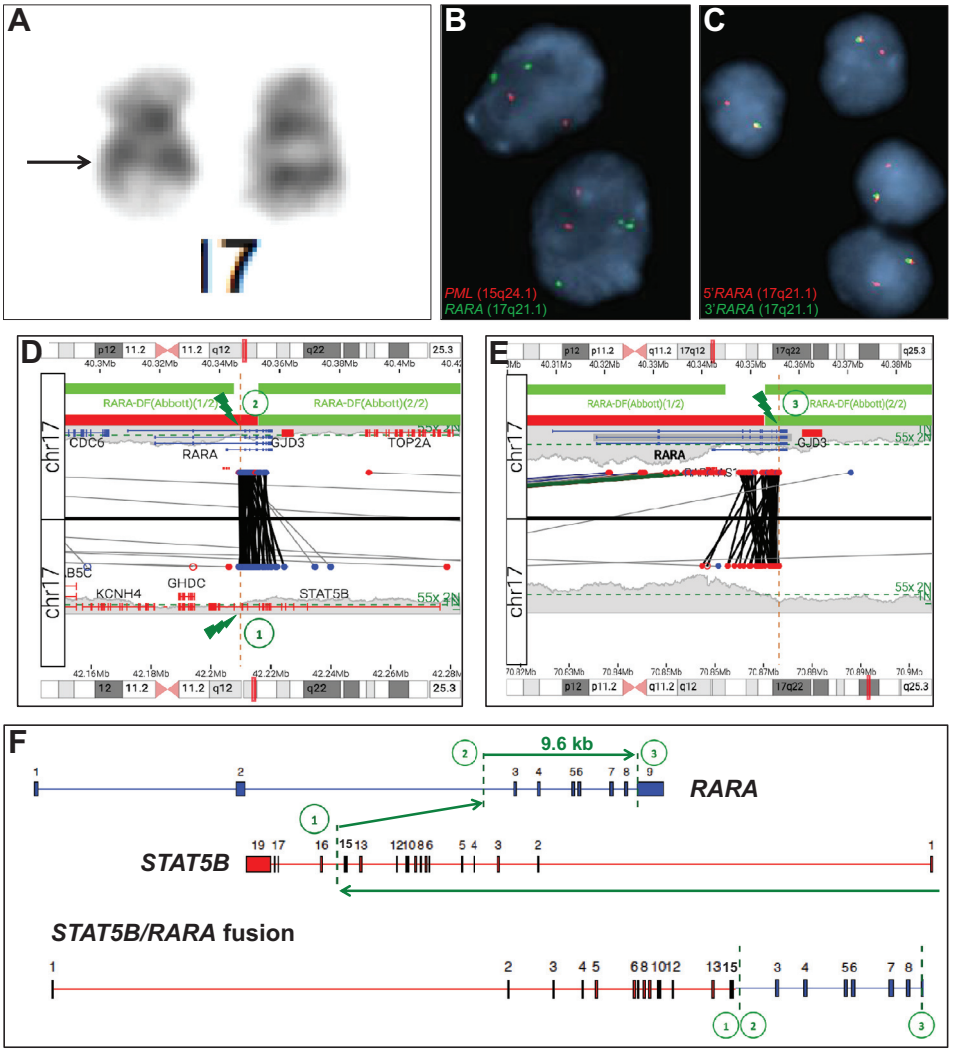

Fig. 2 Genomic findings from the diagnostic bone marrow aspirate material. (A) Partial karyogram demonstrating inv(17)(q21q25) (arrow). This abnormal clone was observed in 15 of 20 metaphases. (B**)** Representative interphase nuclei hybridized with the *PML/RARA* dual-color dual-fusion (D-FISH) probe set showing two red and green signals each, indicating a normal *PML/RARA* D-FISH study. Atypical *RARA* rearrangements by the *PML/RARA* D-FISH probe set are often indicated by an extra *RARA* (green) signal. (C) Representative interphase nuclei hybridized with the RARA break-apart probe (BAP) set showing a single fusion signal (representing an intact *RARA* gene region) and a single red signal (5′*RARA* BAP). Loss of the 3′*RARA* BAP (green signal) was observed in 95% of 200 interphase nuclei and indicates an atypical *RARA* rearrangement. (D) Junction plot demonstrating breakpoints located within the *RARA* gene (intron 2, NM_000964) and the *STAT5B* gene (intron 15, NM_012448). (E) Junction plot demonstrating breakpoints located within the same *RARA* allele (exon 9, NM_000964) involved in the previously described inversion, and an intergenic region located within 17q24.3. (F) View of the *STAT5B/RARA* gene fusion, including exons and inversion breakpoints. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to ATRA therapy [1–3]. The majority of APL (~98%) results from t(15;17)(q24.1;q21.2) (*PML/RARA* gene fusion) that is readily detected by conventional chromosome analysis [1,2]. However, considering the clinical urgency (e.g., disseminated intravascular coagulation) of *PML/RARA* gene fusion detection, the use of *PML/RARA* D-FISH and/or *RARA* BAP FISH studies to detect potential variant *RARA* gene fusion partners that may be resistant to ATRA therapy (*e.g., ZBTB16* and *STAT5B*), are often employed to provide results within 24-hours of specimen receipt [1,3]. While the *PML/RARA* D-FISH probe set is specific for the *PML/RARA* gene fusion, the presence of a single extra *RARA* signal may indicate an atypical *RARA* rearrangement as illustrated by Singh et al. [4]. Once an atypical *RARA* rearrangement is suspected by *PML/RARA* D-FISH, a *RARA* BAP is typically performed for confirmatory purposes. However, given that the *RARA* BAP cannot precisely define the *RARA* gene fusion partner, metaphase FISH or PCR-based assays that target specific *RARA* rearrangements (if clinically available) could be employed for additional information. Utilizing an NGS-based assay like MPseq, most structural abnormalities throughout the genome can be fully characterized with significantly improved accuracy and precision when compared to conventional chromosome and FISH studies [5–7]. The use of MPseq in our case identified a *STAT5B/RARA* fusion resulting from a cryptic inv(17)(q21.2q21.2), in addition to inv(17)(q21.2q24.3) that

was also identified by conventional chromosome studies. Importantly, other methodologies such as real-time quantitative polymerase chain reaction can be utilized to detect and monitor rarely described *RARA* gene fusions [8].

The *STAT5B/RARA* gene fusion is an exceedingly rare variant *RARA* rearrangement in APL that is insensitive to ATRA therapy, the majority having been reported in young adult males and all cryptic by conventional chromosome analysis [1–3,9–14]. Interestingly, Kluk et al. also characterized a similar complex intrachromosomal 17q rearrangement resulting in *STAT5B/RARA* fusion using an NGS-based assay that produced identical *PML/RARA* D-FISH and *RARA* BAP FISH results as described in our case [10]. Indeed, loss of exon 9 (indicated by NGS results and loss of the 3′ *RARA* BAP in both cases) removes a segment of the retinoic acid binding domain coding sequence and spans previously reported deletions associated with ATRA resistance [15]. Thus, these findings may indicate a potential recurrent *STAT5B/RARA* rearrangement mechanism only realized by NGS-based methodologies.

The detection of diagnostic, prognostic and/or targetable genomic abnormalities is essential for multiple hematologic neoplasms, including APL. Traditional cytogenetic methodologies, including conventional chromosome and FISH studies, are usually sufficient for the detection of t(15;17)(q24.1;q21.2), *PML/RARA* fusion in APL. However, atypical *RARA* rearrangements can be difficult to characterize by chromosome and FISH studies alone, and may require the use of more sophisticated methodologies, including NGS-based assays. As we have illustrated in our case, MPseq is capable of detecting structural abnormalities with significantly increased precision and accuracy compared to traditional methodologies, thus enabling the detection of variant *RARA* rearrangements of clinical importance. Cytogeneticists, pathologists and oncologists need to be aware that NGS technology is available to further characterize structural abnormalities beyond the resolution of conventional chromosome and FISH methodologies.

Patient follow-up

Approximately 30 h after admission the patient developed respiratory distress and was transferred to the intensive care unit. Platelet transfusion, fresh frozen plasma, cryoprecipitate, and vitamin K were administered to treat disseminated intravascular coagulation syndrome. ATRA was stopped as he was unable to swallow pills due to respiratory intubation and for the possibility of differentiation syndrome. Two-days after presentation his renal and liver function deteriorated rapidly, and soon after he developed multiple organ failure. With no hope of meaningful recovery, the family decided to withdraw care and the patient expired 3.5 days after presentation to the hospital.

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Conflict of Interest

GV: Algorithms described in this manuscript for mate-pair sequencing are licensed to Whole Genome LLC owned by GV.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.06.007.

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