

CASE REPORT



A *RUNX1::RUNX1T1* AML with a simultaneous false positive *KMT2A* rearrangement: FISH interpretation pitfalls

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ABSTRACT

Introduction: *KMT2A* rearrangement (*KMT2Ar*) is a common genomic alteration in acute leukemia that can be effectively targeted by menin inhibitors. While FISH is the standard laboratory test for *KMT2Ar*, false positives can occur.

Case Report: We present a case of AML in which both *RUNX1::RUNX1T1* and *KMT2Ar* were identified by karyotype analysis and FISH. Although a targeted RNA next generation sequencing (NGS) assay confirmed the presence of the *RUNX1::RUNX1T1* fusion, it did not detect a *KMT2A* fusion transcript. To investigate the discrepancy between the positive *KMT2A* FISH result and the negative fusion transcript, we performed whole-genome mate-pair DNA NGS to examine the *KMT2A* locus on chromosome 11q23. This analysis revealed a breakpoint located 5.8 kb downstream of *KMT2A*, which did not disrupt the gene itself. Given that *KMT2A* FISH probes cover approximately 1 Mb around *KMT2A*, this subtle shift led to a split-apart signal pattern mimicking a genuine *KMT2A* rearrangement, resulting in a false positive FISH interpretation.

Conclusion: This case highlights a false positive *KMT2Ar* in primary AML, indicating the need for additional molecular testing for confirmation.

ARTICLE HISTORY

Received 2 July 2024

Accepted 18 October 2024

KEYWORDS

KMT2A; *RUNX1*; *RUNX1T1*; FISH

Background

Lysine methyltransferase 2A (*KMT2A*), also known as mixed-lineage leukemia (*MLL*), is rearranged in approximately 80% of infant B-cell acute lymphoblastic leukemia (B-ALL) and about 5-15% of children and adults with acute leukemia of lymphoid, myeloid, or mixed-cell lineage [1]. Various fusion partners impact the prognosis for *KMT2Ar* leukemia. Additionally, cytogenetic abnormalities are linked to poorer overall survival (OS) in pediatric acute myeloid leukemia (AML) [2]. In adults, mutations in *DNMT3A*, *KRAS*, and *TP53* are associated with reduced OS [3]. Furthermore, minimal residual disease (MRD) status after induction therapy serves as an independent prognostic factor [4].

KMT2A plays a pivotal role in transcriptional regulation by inducing the trimethylation of lysine 4 on histone 3, and its rearrangement in leukemia leads to the dysregulation of oncogenes and tumor suppressors [5]. Menin interacts with *KMT2A* and its associated proteins, including *WDR5* and *ASH2L*, to stabilize *KMT2A* complexes. The menin inhibitor Revumenib blocks the binding of the *KMT2A* to its target genes such as homeobox (*HOX*) genes and their DNA-

binding cofactor MEIS1 (*HOX/MEIS*), proving effective in treating *KMT2Ar* leukemia [6,7]. A phase 2 clinical trial of Revumenib on relapsed or refractory acute leukemia with *KMT2Ar* or *NPM1* mutation (with similar *HOX/MEIS* overexpression) (NCT04065399) showed complete remission or complete remission with partial hematologic recovery (CR/CRh) in 30% of patients, with 78% of this group achieving undetectable measurable residual disease (MRD) via multiparameter flow cytometry. The overall response rate was 53%, with a median time to CR/CRh of 1.9 months [8]. Moreover, a preclinical study indicated that JNJ-75276617, a novel menin-*KMT2A* inhibitor, demonstrates anti-proliferative activity in leukemia with *KMT2Ar* or *NPM1* alterations, as well as in cell lines harboring revumenib-resistant mutants (*MEN1*^{M327I} and *MEN1*^{T349M}) [9].

Given Revumenib's success in treating *KMT2Ar* leukemia, the accurate testing of *KMT2Ar* becomes increasingly critical, often performed through FISH assay in clinical laboratories. Herein, we report a false positive *KMT2Ar* by FISH in a primary AML with *RUNX1::RUNX1T1*. Through literature review, several

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cases with potential false positive or nonproductive *KMT2Ar* were identified [10–15], suggesting a chromosome fragile site near *KMT2A* that complicates the interpretation of *KMT2A* testing [16].

Case presentation

A male in his late thirties presented with a fever and cough for 10 days. The complete blood counts showed a white blood cell count of $33.08 \times 10^9/L$, hemoglobin 54 g/L, platelet count of $22 \times 10^9/L$, and neutrophil count of $13.12 \times 10^9/L$. The bone marrow smear showed 31% blasts with immature chromatin, 1–3 prominent nucleoli, and bluish cytoplasm (Figure 1A). Blasts were stained positive for peroxidase (POX) (100%). Bone marrow flow cytometry demonstrated 33% blasts with a myeloid immunophenotype. These blasts were positive for CD34, CD13, CD33, CD38, CD117, CD56, HLA-DR, and CD19 (weak), while negative for CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD14, CD15, CD20, CD36, and CD64. A diagnosis of AML was made. Karyotype analysis from unstimulated bone marrow showed a loss of chromosome Y and a three-way translocation involving chromosomes 8,

11, and 21 (45,X,-Y,t(8;11;21)(q22;q23;q22)) (Figure 1B). Because these chromosome breakpoints could target *RUNX1*, *RUNX1T1* or *KMT2A*, FISH was done on abnormal metaphases, which was positive for both the *RUNX1::RUNX1T1* and *KMT2A* rearrangement (Figure 1C and 1D). A simultaneous targeted RNA NGS assay of 87 fusion genes associated with hematopoietic malignancy confirmed the *RUNX1::RUNX1T1* fusion (Figure 2A), however, *KMT2A* fusion transcript was not detected.

To address the discrepancy between a positive *KMT2A* FISH result and a negative fusion transcript, we checked the breakpoint of the *KMT2A* locus at chromosome 11q23 by performing a whole-genome mate-pair DNA NGS, which identified a chromosome 11 breakpoint located 5.8 kb downstream of *KMT2A*, with no disruption of the *KMT2A* gene itself (Figure 2B–D). Given that *KMT2A* FISH probes encompass approximately 1 Mb of the genomic region surrounding *KMT2A* (Figure 2E), the subtle shift of the breakpoint resulted in a split-apart signal pattern resembling genuine *KMT2A* rearrangements, leading to a false positive interpretation of the *KMT2A* FISH test (Figure 1D). The assay also confirmed the presence

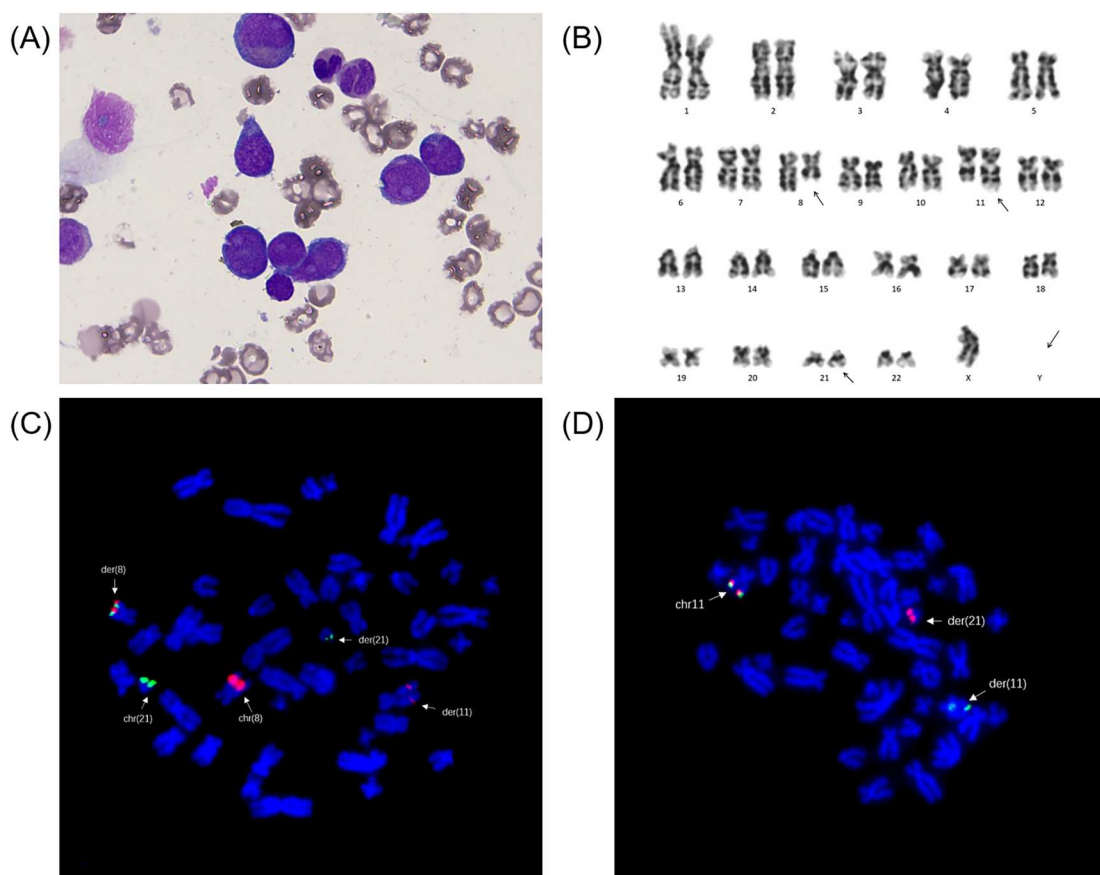


Figure 1. (A) The bone marrow aspirate revealed blasts of immature chromatin, 1–3 prominent nucleoli, and bluish cytoplasm. (B) Karyotypic analysis of unstimulated bone marrow specimen showed a 3-way translocation involving chromosomes 8, 11 and 21 and a loss of chromosome Y (arrows). (C) Metaphase FISH with a dual fusion probe set confirmed the presence of the *RUNX1::RUNX1T1* fusion, with green signals indicating *RUNX1* and red signals indicating *RUNX1T1* (HealthCare, China). (D) Metaphase FISH with a split-apart *KMT2A* probe set revealed a *KMT2A* rearrangement, with the 5' *KMT2A* (green signal) remaining on chromosome 11q23 while the 3' *KMT2A* (red signal) relocating to chromosome 21q21 (HealthCare, China).

of a chromosome 8 breakpoint within the intron 1 of *RUNX1T1* and a chromosome 21 breakpoint within the intron 5 of *RUNX1*, resulting in an intact reading frame of the *RUNX1::RUNX1T1* fusion (Figure 2B). To

assess the impact of the chromosome 11 breakpoint on *KMT2A* expression, we compared the expression levels of *KMT2A* in our case with those of two non-neoplastic bone marrow samples, two AML cases with

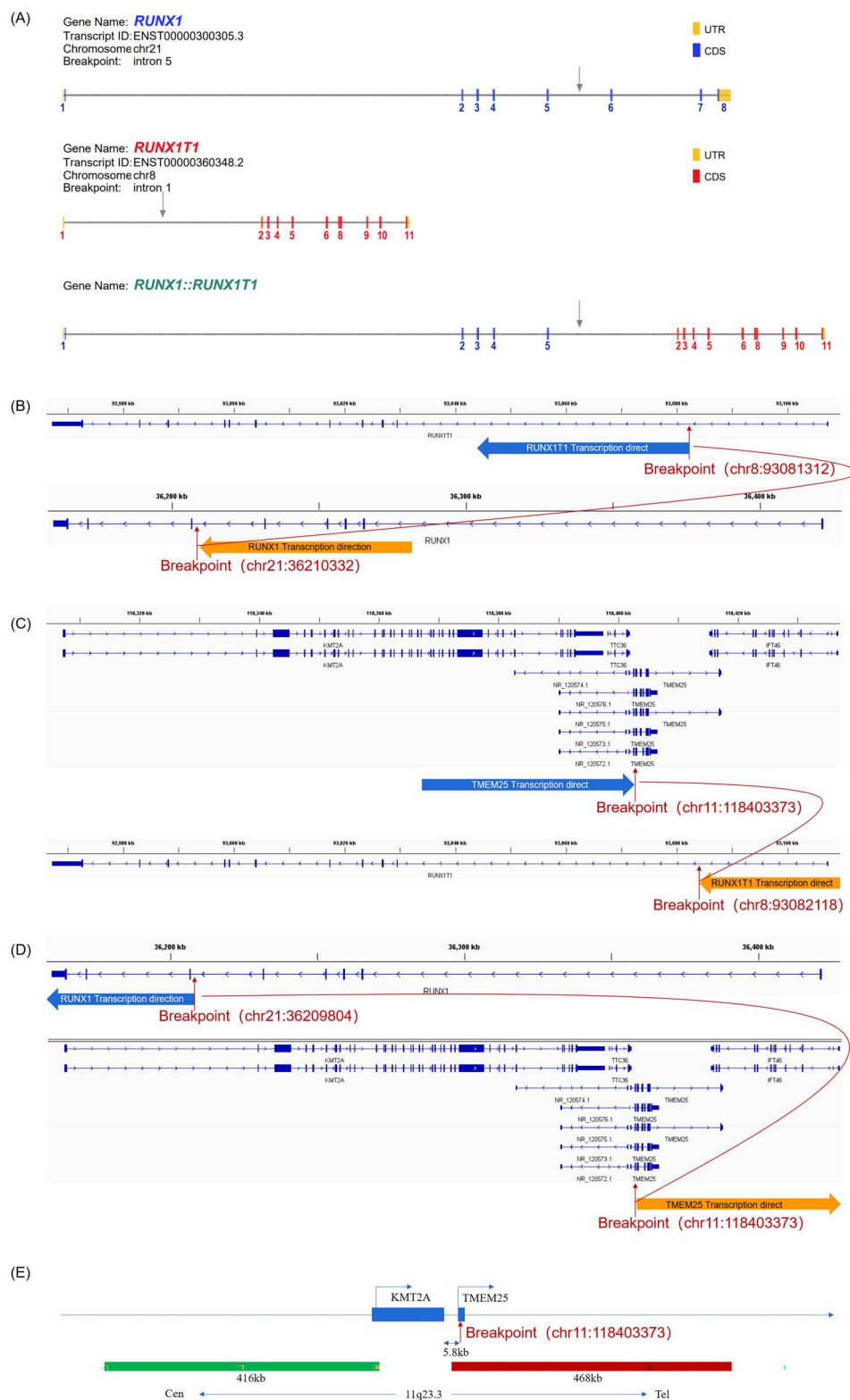


Figure 2. (A) RNA NGS analysis of bone marrow aspirates confirmed the presence of the *RUNX1::RUNX1T1* fusion, with arrows marking the breakpoints of *RUNX1* and *RUNX1T1*. (B-D) Whole-genome mate-pair sequencing mapped the genomic breakpoints of the 3-way translocation t(8;11;21). The fusion breakpoints between chromosomes 8 and 21 (B), chromosomes 8 and 11 (C), and chromosomes 21 and 11 (D) were indicated by arrows, with genomic coordinates provided. (E) The *KMT2A* FISH probe set is located on chromosome 11q23, with green representing 5'*KMT2A* and red representing 3'*KMT2A*. The chromosome 11q23 breakpoint is outside of the *KMT2A* and was marked by an arrow.

KMT2Ar (AML-*KMT2Ar*), after normalization with β -actin (*ACTB*). The analysis revealed no significant differences in *KMT2A* expression between any two groups (data not shown). Furthermore, *HOXA9* expression, a well-known target of *KMT2A* fusion proteins [6], was evaluated. While two cases with *KMT2Ar* showed the expected increase in *HOXA9* expression, there was minimal *HOXA9* expression in the normal bone marrow or in the case with a false positive *KMT2Ar* (data not shown).

In addition to the *RUNX1::RUNX1T1*, a targeted DNA NGS assay of 72 AML-associated genes found several gene mutations, including *ASXL1* p.Q1286* (variant allele frequency (VAF) of 41.5%), *KIT* p.D816 V (5.3%), *JAK2* p.V617F (17.5%). The patient underwent 7 + 3 chemotherapy, receiving cytarabine at 100 mg/m² from days 1–7 and idarubicin at 10 mg/m² from days 1–3. A bone marrow aspirate on day 10 showed 6% blasts, indicating a partial response. By day 28, he achieved morphologic CR with a MRD level of 1.8×10^{-3} by flow cytometry. The patient continued two cycles of high-dose cytarabine (1 g/m² twice daily on days 1–3) and mitoxantrone at 10 mg on day 4. On day 28 after these cycles, the MRD level was 3.6×10^{-4} . He then proceeded with two additional cycles of even higher-dose cytarabine (2 g/m²), achieving an MRD level of 2.4×10^{-4} on day 28 after these treatments. Finally, the patient received azacitidine and venetoclax, followed by myeloablative conditioning (mBUCY) and allogeneic hematopoietic stem cell transplantation. He continues to be monitored through regular follow-ups.

Discussion

We documented a case involving a false positive *KMT2Ar* in a primary AML with *RUNX1::RUNX1T1*. While secondary gene fusions can occur, the consensus is that only one fusion is typically present in primary leukemia [10]. A large cohort study of 8226 leukemia patients found that only 0.3% harbored double fusions, often correlating with disease progression [11]. Thus, the concurrent presence of both *RUNX1::RUNX1T1* and *KMT2Ar* in our patient raised concerns, prompting further investigation. Subsequent analysis revealed the *KMT2Ar* as a false positive due to a breakpoint closely situated to the *KMT2A* gene but without *KMT2A* interruption. These findings emphasize the necessity of confirming the presence of a functional fusion through molecular testing, such as RNA NGS, DNA NGS, or reverse transcription-polymerase chain reaction (RT-PCR), in specific clinical scenarios. Alternatively, a more effectively designed *KMT2A* probe set could help minimize false positive results in FISH testing.

Two similar false *KMT2Ar* B-ALL was recently reported [14,15]. In one of the cases, a 3-year-old

female showed a classic *ETV6::RUNX1* rearrangement via FISH assay, along with an additional *KMT2A::MLL3* fusion detected by both karyotype and FISH. However, further investigation by whole-genome sequencing (WGS) confirmed the false positive *KMT2A::MLL3* fusion caused by a complex genomic rearrangement involving these regions. In another case, the 7-year-old female showed hyperdiploid karyotype (56 chromosomes) and *KMT2Ar* by FISH, but with no productive fusion identified through target fusion panel and whole transcriptomic sequencing. Subsequent RNA expression analyses confirmed the hyperdiploid classification. Additionally, three cases of B-ALL with concurrent *ETV6::RUNX1* rearrangement and *KMT2Ar* were documented [12,13]. However, a functional *KMT2A* fusion transcript was not evaluated in these cases. These cases all exhibited comparable clinical presentations and therapeutic responses to classic B-ALL with *ETV6::RUNX1* rearrangement. Therefore, the possibility of a false positive *KMT2Ar* cannot be ruled out.

KMT2Ar represents one of the most common events in tumors, with over 100 fusion partners identified [17,18]. Intriguingly, *KMT2A* is located on chromosome 11q23, where a chromosome-fragile site resides [16]. Further investigation into whether *KMT2Ar*, including both functional and non-functional rearrangements, are associated with the chromosome fragile site warrants consideration.

In summary, we present another case of false positive *KMT2Ar*. Given the potential efficacy of menin inhibitors in targeting this rearrangement, cautious interpretation of positive FISH results for *KMT2Ar* is imperative and may necessitate further validation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

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