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MLL-SEPT5 Fusion Transcript in Two *de novo* Acute Myeloid Leukemia Patients With t(11;22)(q23;q11)

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Dear Editor,

Rearrangements involving mixed lineage leukemia (MLL) are common chromosome aberrations in infant, pediatric and adult acute leukemia, which are generally associated with poor prognosis. To date, more than 79 partner genes have been identified [1]. *MLL* fusion partners can be classified into four groups: nuclear proteins (*MLLT3*, *MLLT10*, and *MLLT1*), cytoplasmatic proteins (*GAS7*, *SH3GL1*, *EPS15*, and *MLLT4*), histone acetyltransferases (*EP300*, *CREBBP*), and the septin family (*SEPT2*, *SEPT5*, and *SEPT6*) [2]. In pediatric and adult AML, the most frequent fusion partners are represented by *MLLT3-AF9* (9p22), *MLLT10-AF10* (10p12), *ELL* (19p13.1), *MLLT4-AF6* (6q27), and *MLLT1-ENL* (19p13.3) [1, 3]. However, as a partner gene, the *SEPT5* gene has been reported in only five AML cases (Table 1).

Here we present two cases of *de novo* AML with *MLL-SEPT5* transcript. Chromosomal analysis revealed a karyotype of 46, XX/XY, t(11;22)(q23;q11.2). Reverse transcription polymerase chain reaction (RT-PCR) analysis indicated an *MLL-SEPT5* fusion transcript. Sanger sequencing of the PCR product confirmed the fusion between *MLL* and *SEPT5*. Notably, a new fusion transcript between *MLL* exon 8 and *SEPT5* intron 2 was detected in one of the patients.

Patient 1 was a 21-yr-old man who presented with fever, oral ulcer, and tonsillitis. His hemoglobin level was 8.5 g/dL, white blood cell count was 35.45×10^9/L, and platelet count was 29×10^9/L. Bone marrow aspiration was hypercellular, containing 81% blasts. Immunophenotypic analysis showed that the blasts were positive for CD45, CD33, CD117, CD15, and HLA-DR, but negative for other markers. Cytogenetic studies revealed the karyotype of 46, XY, t(11;22)(q23;q11.2) [8]/46,XY [2]. Fluorescence *in situ* hybridization analysis using a MLL-specific probe showed a split in the *MLL* gene (Fig. 1). He received three courses of induction chemotherapy in our hospital followed by a mother-to-son haploidentical bone marrow transplantation. To date, he is in complete remission.

To validate *MLL-SEPT5* existence, we performed RT-PCR and direct DNA sequencing. Total RNA was extracted from the bone marrow cells using TRIzol reagent (Invitrogen, Paisley, UK) and chloroform. cDNA was obtained by RT-PCR using the M-MLV reverse transcriptase (Promega, Madison, WI, USA). The resulting cDNA was amplified by PCR using 2×Hieff PCR Master Mix (Yeason, Shanghai, China) and the following primers: 5′-GCTCCACCCATCAAACCAAT-3′ from exon 5 of *MLL* and 5′-TTCTTCTCAATGTCCACCGT-3′ from exon 4 of *SEPT5* (Fig. 2). PCR products were sequenced on both strands by using an Applied Biosystems ABI 3730 XL DNA analyzer (Thermo Fisher Scientific,

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Table 1. Reported cases of *de novo* AML with *MLL-SEPT5* fusion

Patient	Age/sex	Diagnosis	*MLL-SEPT5* fusion transcript	References
1	11.5 mo/F	AML with maturation	*MLL* exon 7-*SEPT5* exon 3	Megonigal *et al*., 1998 [8]
2	13 mo/F	AML without maturation	*MLL* exon 7-*SEPT5* exon 3	Megonigal *et al*., 1998 [8]
3	39 yr/M	AML with maturation	*MLL* exon 6-*SEPT5* exon 4	Tatsumi *et al*., 2001 [5]
4	23 mo/F	Acute monocytic leukemia	*MLL* exon 10-*SEPT5* exon 3	Launay *et al*., 2014 [9]
5	32 yr/M	AML with maturation	*MLL* exon 10-*SEPT5* exon 3	Gao *et al*., 2014 [10]
6	21 yr/M	Acute monocytic leukemia	*MLL* exon 8-*SEPT5* intron 2	Present
7	22 yr/F	Acute monocytic leukemia	*MLL* exon 10-*SEPT5* exon 3	Present

Abbreviations: F, female; M, male; mo, month; MLL, mixed lineage leukemia.

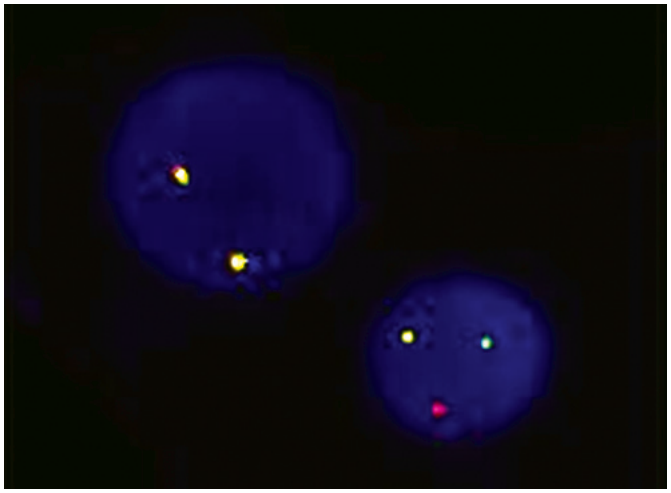

Fig. 1. FISH analysis using 11q23 break-apart probe revealed *MLL* rearrangement. Separation of a green and a red signal was observed.
Abbreviation: MLL, mixed lineage leukemia.

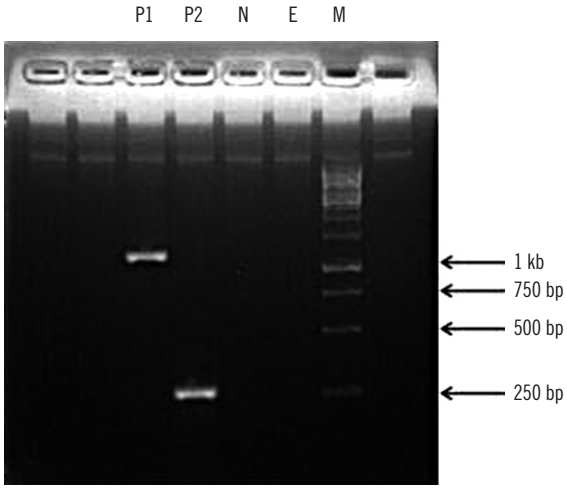

Fig. 2. Identification of the *MLL-SEPT5* fusion transcripts by reverse transcription-PCR. Lane M, 1kb DNA ladder; Lane E, empty control; Lane N, PCR control; Lane P1, patient 1; Lane P2, patient 2.
Abbreviation: MLL, mixed lineage leukemia.

Waltham, MA, USA). The sequence was analyzed with Chromas Lite software (version 2.01, Technelysium, Brisbane, Australia). The fusion between *MLL* exon 8 and *SEPT5* intron 2 is indicated by the chromatogram (Fig. 3A). Both breakpoints in the two genes have not been reported previously.

Patient 2 was a 22-yr-old woman who presented with sore throat, dysphagia, fever, and asthenia. Her hemoglobin level was 11.2 g/dL, platelet count was 54×10^9/L, and white blood cell count was 140×10^9/L, with more than 89% blasts in peripheral blood. Bone marrow smears showed hypercellularity, containing 95.5% blasts. Flow cytometric analysis of the blasts showed the following immunophenotype: CD45, CD14, CD13, and CD33. Karyotype was 46, XX, t(11;22)(q23;q11.2) in all 10 cells examined. She did not receive chemotherapy in our hospital.

MLL-SEPT5 fusion was verified by the previously used method and the following PCR primers: 5′-CCGGTCAATAAGCAGGAGAA-3′ from exon 10 of *MLL* and 5′-CAGCCATGAGTGTGAAG-3′ from exon 3 of *SEPT5*. Long distance inverse-PCR is very useful for recognizing unusual cryptic cytogenetic findings, such as a rare *MLL* partner gene, or discrepancies in molecular results that are hard to identify through common molecular diagnostic methods [4]. Since we have identified the partner gene from previous studies, we utilized RT-PCR and Sanger DNA sequencing to confirm the fusion gene. The results indicate the rearrangement between *MLL* exon 10 and *SEPT5* exon 3 (Fig. 3B).

We described two new cases of *de novo* AML with t(11;22)(q23;q11.2), resulting in a *MLL-SEPT5* fusion. To date, five such cases have been reported, including three infants and two adults (Table 1). All patients with the *MLL-SEPT5* fusion gene were diagnosed with AML. Tatsumi *et al*. [5] examined *SEPT5* expression in 27 leukemia cell lines, including 13 AML and 14

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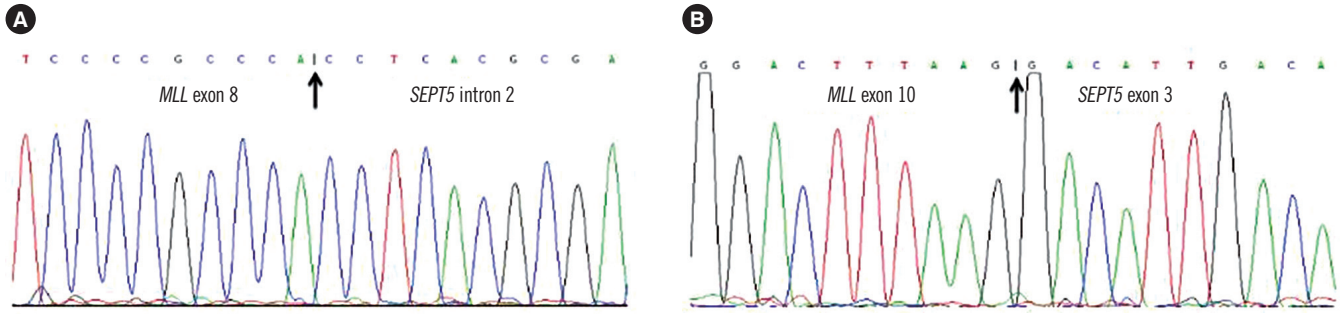

Fig. 3. Sequences of junction of the *MLL-SEPT5* chimeric transcript. (A) Patient 1, (B) patient 2.
Abbreviation: MLL, mixed lineage leukemia.

ALL cell lines, which indicated that *SEPT5* expression in AML cell lines was significantly higher than that in ALL. *MLL-SEPT5* fusion gene was important in the leukemogenesis of AML with t(11;22)(q23;q11.2).

As previously reported, reciprocal *MLL* fusion proteins may have an important role in leukemia development [6, 7]. The *MLL-SEPT5* fusion transcript was uncommon and leukemogenesis mechanisms of the fusion protein remain poorly understood. Future studies will be required to elucidate these mechanisms.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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