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Persistent detection of a novel *MLL-SACM1L* rearrangement in the absence of leukemia

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Abstract

Most chromosomal rearrangements including the mixed lineage leukemia (*MLL*) gene are manifested as leukemia and predict a poor prognosis. Although more than 50 *MLL*-rearrangement partners are characterized, *MLL*-related leukemogenesis remains to be understood. Here we report a case of a 3-year-old boy bearing a novel *MLL*-rearrangement with the suppressor of actin mutations 1-like (*SACM1L*) gene in the absence of leukemia. Bone marrow cells harboring the *MLL-SACM1L* rearrangement appeared during chemotherapy for acute lymphoblastic leukemia with hyperdiploidy and were continuously detected over 7 years without clonal expansion.

Key words: Acute lymphoblastic leukemia, chemotherapy, *MLL*, *SACM1L*

Introduction

The mixed lineage leukemia (*MLL*) gene, also known as acute lymphoblastic leukemia-1 (*ALL1*) gene or human trithorax (*HRX*) gene, is localized on chromosome 11q23, consists of 36 exons, and encodes a histone methyltransferase implicated in epigenetic regulation of gene expression that is critical for normal embryonic development and hematopoiesis. Chromosomal rearrangements involving the *MLL* gene are frequently detected in infant leukemia and therapy-related acute myeloid leukemias (t-AML) that develop in patients treated with topoisomerase II inhibitors for other malignancies, and predict a poor prognosis [1].

Most *MLL*-rearrangements map to an 8.3-kb breakpoint cluster region (BCR) spanning *MLL* exons 8-14, and result in the generation of a chimeric gene fusing 5'-portion of *MLL* with 3'-portion of a partner. We previously reported the *AF3p21/NCKIPSD* gene, also known as SH3 protein interacting with Nck 90 kDa (SPIN90) or Wiskott-Aldrich syndrome protein interacting SH3 protein (WISH), as a *MLL*-rearrangement partner in t-AML with t(3;11)(p21;q23) [2]. Now more than 50 different *MLL*-rearrangement partners have been identified, and *AF4*, *AF6*, *AF9*, and *ENL* are emerged as common *MLL*-rearrangement partners

in acute lymphoblastic leukemia (ALL) and AML. Although the proteins associated with MLL and the downstream mediators of MLL transcriptional regulations are identified in addition to *MLL*-rearrangement partners, the molecular mechanism of *MLL*-related leukemogenesis remains to be understood. Here we have characterized a novel *MLL*-rearrangement in a case of a 3-year-old boy with t(3;11)(p21;q23) during chemotherapy for pre B-cell ALL.

Patient and methods

A 3-year-old boy was admitted to our hospital because of high fever and anemia. The patient gave written informed consent and was treated in accordance with the Guidelines for Clinical Research of Kobe University Graduate School of Medicine. The peripheral blood tests showed hemoglobin 4.8 g/dl, platelets 10 x 10^9/L, and white blood cell 17.8 x 10^9/L with 3% mature granulocyte, 20% lymphocytes, and 77% blasts. The bone marrow aspirates showed a hypercellularity of nucleated cell 38.6 x 10^{10}/L with 0.4% myeloid cells, 0.8% erythroid cells, 1.2% lymphoid cells, 0.2% monocytes, and 96.8% blasts. The blasts were positive for CD19, CD10, CD20, CD22, CD34, HLA-DR, and cytoplasmic μ. Cytogenetic analysis of bone marrow cells showed a hyperdiploid karyotype with 58<2n>, XY, +X, +4, +6, +10, +15, +17p, +18, +18, +20, +21, +21, +22 (Table 1 and Fig. 1). The patient was diagnosed with pre B-cell ALL and was treated according to the Japanese Association of Childhood Leukemia Study (JACLS) ALL-97 Chemotherapy protocol. The therapy was completed at 26 months, and the patient maintains event-free survival at present.

Although the bone marrow aspirates indicated that the patient remained in complete remission, cytogenetic analysis revealed t(3;11)(p21;q23), which was detected in 3/20 metaphase cells at 18 months after diagnosis and continued to be detected until 40 months after diagnosis (Table 1 and Fig. 2A). An *MLL*-split signal analyzed by fluorescence in situ hybridization (FISH) with *MLL* dual color break apart rearrangement probes (Vysis, Downers Grove, IL), which cover a centromeric 350-kb portion and a telomeric 190-kb portion of the *MLL* gene BCR, was observed in 16/100 interphase cells at 26 months after diagnosis and

continued to be observed throughout the follow-up period (Table 1 and Fig. 2B). The cumulative doses of the anti-leukemia agents used before the identification of *MLL*-rearrangements were 1,000 mg/m^2 etoposide, which was less than the dose recommended by the JACLS protocol because of repeated severe sepsis, 7,800 mg/m^2 cyclophosphamide, and 300 mg/m^2 THP-adriamycin.

To identify the *MLL*-rearranged transcript, total RNA was isolated from bone marrow cells at 95 months after diagnosis with Isogen (Wako, Osaka, Japan) and subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) using Long Range 2-step RT-PCR kit (Qiagen, Valencia, CA) with *MLL* exon 7 (5'-GAAAAGAAGTTCCCAAAACCAC-3'; 5'-AAGCAGCCTCCACCACCAGA-3'), *AF3p21/NCKIPSD* exon 5 (5'-GAGGCTGAGGTTGTACCA-3'; 5'-GCTGGAGCCTGGTTCAGATG-3'), and *SACM1L* intron 1 (5'-TGTATCTTCTCCTGTGCCTACCT-3') primers.

To determine the breakpoints of t(3;11)(p21;q23), genomic DNA was isolated from bone marrow cells at 95 months after diagnosis using Isogen (Wako). The approximately 8-kb *MLL-SACM1L* and 0.3-kb *SACM1L-MLL* junction regions were amplified by PCR using PrimeSTAR GXL DNA polymerase (Takara, Otsu, Japan) with *MLL* intron 7 (5'-GTCAGTACTAAAGTAGTCGTTG-3'), *MLL* intron 10 (5'-TGCTACTCTAATAGCAGATTCCTTC-3'), *SACM1L* intron 1 (5'-TGTATCTTCTCCTGTGCCTACCT-3'), and *SACM1L* intron 4 (5'-TTTCATACACACTCCGAGCAA-3') primers.

Results

In addition to our previous case of t-AML with an *MLL-AF3p21/NCKIPSD* rearrangement [2], two cases of hematopoietic malignancy with t(3;11)(p21;q23), duodenal malignant lymphoma and refractory anemia, were reported without determining their genomic break points [3, 4]. To identify an *MLL*-rearrangement partner in our present case, we first

searched for *MLL-AF3p21/NCKIPSD* chimeric transcripts. When we performed nested RT-PCR using *MLL* exon 7 and *AF3p21/NCKIPSD* exon 5 primers, a single band of unexpected size was detected. Surprisingly, its DNA sequencing revealed that the sequence rearranged with the *MLL* gene matched with the intron 1 sequence of the suppressor of actin mutations 1-like (*SACM1L*) gene, also known as a human homologue of yeast suppressor of actin 1 (SAC1), not the exon 5 sequence of the *AF3p21/NCKIPSD* gene. The *SACM1L* gene was located at 3-Mb distant from the *AF3p21/NCKIPSD* gene on 3p21. Given that 14/20 residues of the *MLL* exon 7 primer were identical to the sequence located between nucleotide positions 47,038 and 47,057 of *SACM1L* (numbered according to GenBank accession no. AJ297357), we then looked for *MLL-SACM1L* chimeric transcripts. RT-PCR using *MLL* exon 7 and *SACM1L* intron 1 primers produced a single band of an expected size. Its DNA sequencing demonstrated that *MLL* exon 9 was fused to *SACM1L* intron 1. The resulting transcript was predicted to encode a truncated MLL protein containing *MLL* exons 1-9 and eight amino acids from *SACM1L* (Fig. 3A).

To determine the genomic breakpoints of t(3;11)(p21;q23), we amplified the junction region of *MLL-SACM1L* by PCR using *MLL* intron 7 and *SACM1L* intron 1 primers. Sequencing of the resulting 8-kb product revealed that the genomic breakpoints were located in exon 10 of *MLL* at nucleotide position 4,312 (numbering according to GenBank accession no. NM_005933.2) and in intron 4 of *SACM1L* at nucleotide position 52,705 (numbering according to GenBank accession no. AJ297357) (Fig. 3B). Based on the *MLL* and *SACM1L* breakpoints, the reciprocal *SACM1L-MLL* junction region was amplified by PCR with using *SACM1L* intron 4 and *MLL* intron 10 primers. A 0.3-kb PCR product of an expected size for the reciprocal *SACM1L-MLL* translocation was obtained. Its DNA sequencing confirmed a balanced chromosome translocation with no insertions/deletions of the *MLL* gene and a 3-bp overlap of the *SACM1L* gene (Fig. 3C).

Discussion

The present study adds *SACM1L* to the growing list of *MLL*-rearrangement partners. The

SACM1L gene encodes phosphoinositide phosphatase and is involved in the organization of both Golgi membranes and mitotic spindles within the cell. Although the biological functions of its phosphatase activity remain poorly characterized, the *SACM1L* knockout mouse shows early embryonic lethality [5]. In our present case, the antisense strand of the *SACM1L* gene was rearranged with the *MLL* gene. The transcript contained the *MLL* exon 9 fused to the *SACM1L* intron 1 instead of the partial *MLL* exon 10 fused to the *SACM1L* intron 4 (Fig. 3). This splice site was accurately identified by Shapiro's score, as the former score (98.1) was higher than the latter score (95.6) [6]. The resulting chimeric protein was predicted to contain *MLL* exons 1–9, which included all domains found commonly in the chimeric MLL oncoprotein, and eight amino acids encoded by the *SACM1L* gene.

Although nearly all known *MLL*-rearrangements manifest as leukemia, the *MLL*-rearrangement with *ARHGEF17*, also known as 164 kDa Rho guanine nucleotide exchange factor (p164-RhoGEF), was reported to result in a clonal expansion of bone marrow cells over a 30-months period of complete remission without any leukemogenic sign [7]. The present case provides another example for the *MLL*-rearrangements in the absence of leukemia. However, there is an important difference between the *MLL-ARGF17* rearrangement and the *MLL-SACM1L* rearrangement. Whereas the *MLL-ARGF17* rearrangement confers clonal expansion to the bone marrow cells, the *MLL-SACM1L* rearrangement was persistently detected in the bone marrow cells without clonal expansion.

In AML, a close cooperation has been observed between class I mutations stimulating cell proliferation and class II mutations impairing hematopoietic differentiation [8]. According to this scenario, the *MLL-SACM1L* and *MLL-ARGF17* rearrangements may need an additional class I mutation to cause leukemia. Although the persistent detection of the *MLL-SACM1L* rearrangement over 7 years suggests that the *MLL-SACM1L* rearrangement occurs within the hematopoietic stem cells or confers a self-renewal activity to the committed progenitor cells, it is still premature to discuss further the relationship of the *MLL-SACM1L* rearrangement with the *MLL*-related leukemogenesis.

Conflict of interest

The authors declare no competing financial interests.

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Contribution. TM, DH, KK, KU, TY and AH performed experiments; TM, NN, YK, YT, HN and MM designed research; TM and NN wrote the paper.

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Figure legends

Table 1. Summary of cytogenetic analysis of bone marrow cells. Hyperdiploid indicates the number of cells with a hyperdiploid karyotype in 20 metaphase cells examined. t(3;11)(p21;q23) indicates the number of cells with t(3;11)(p21;q23) in 20 metaphase cells examined. *MLL*-rearrangements indicate the number of cells with split *MLL* signals in FISH analysis in 100 interphase cells examined. Bone marrow indicates the result of morphological analysis of bone marrow cells. m: months. -: not tested. ALL: acute lymphoblastic leukemia. CR: complete remission.

Fig. 1. Cytogenetic analysis of bone marrow cells at diagnosis of pre B-cell ALL. The G-banded karyotype is 58<2n>, XY, +X, +4, +6, +10, +15, +17p, +18, +18, +20, +21, +21, +22.

Fig. 2. Cytogenetic analysis of bone marrow cells at 18 months after diagnosis. (A) The G-banded karyotype is 46, XY, t(3;11)(p21;q23). Arrows indicate rearranged chromosomes. (B) FISH analysis. Red and green signals correspond to 5'-*MLL* and 3'-*MLL* probes, respectively. Thick and thin arrows indicate a split *MLL* signal. Arrowhead indicates an intact *MLL* signal.

Fig. 3. *MLL-SACM1L* rearrangement in t(3;11)(p21;q23). (A) Sequence of a *MLL-SACM1L* transcript. (B) Sequence of a genomic *MLL-SACM1L* junction on 11q23. (C) Sequence of a genomic *SACM1L-MLL* junction on 3p21.

Table 1

Date	Hyperdiploid	t(3;11)(p21;q23)	*MLL*-rearrangement	Bone marrow
Diagnosis	8	0	—	ALL
6 m	0	0	—	CR
18 m	1	3	—	CR
26 m	0	6	16	CR
29 m	0	1	14	CR
33 m	0	6	—	CR
36 m	0	1	7	CR
40 m	0	2	7	CR
47 m	0	0	5	CR
56 m	0	0	—	CR
64 m	0	0	1	CR
70 m	0	0	3	CR
82 m	0	0	0	CR
95 m	0	0	1	CR
102 m	0	0	5	CR

Fig. 1

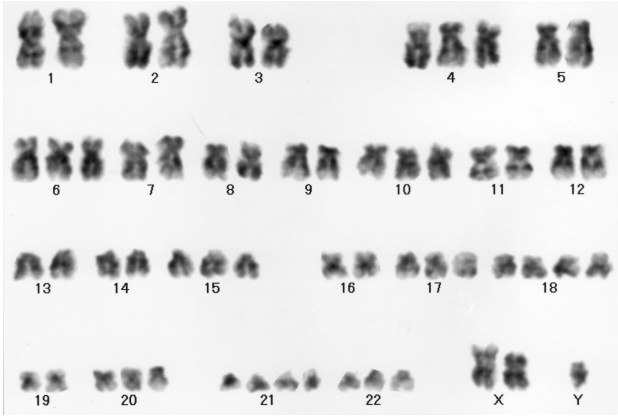
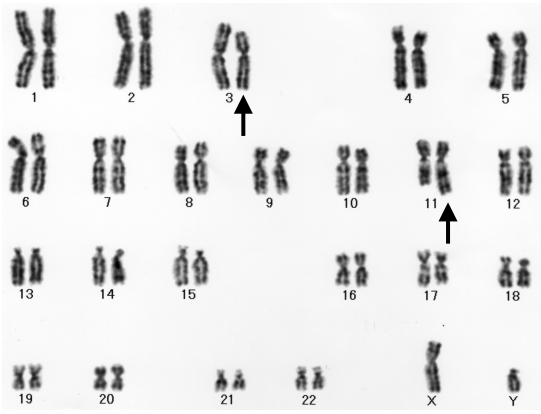


Fig. 2

A



B

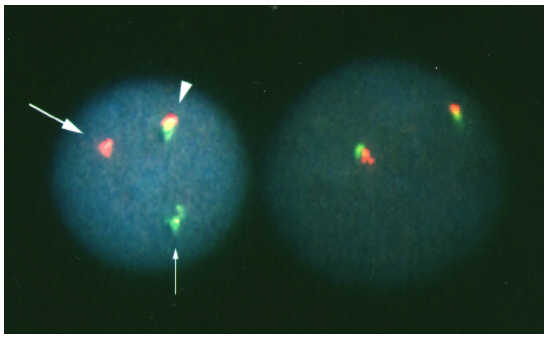
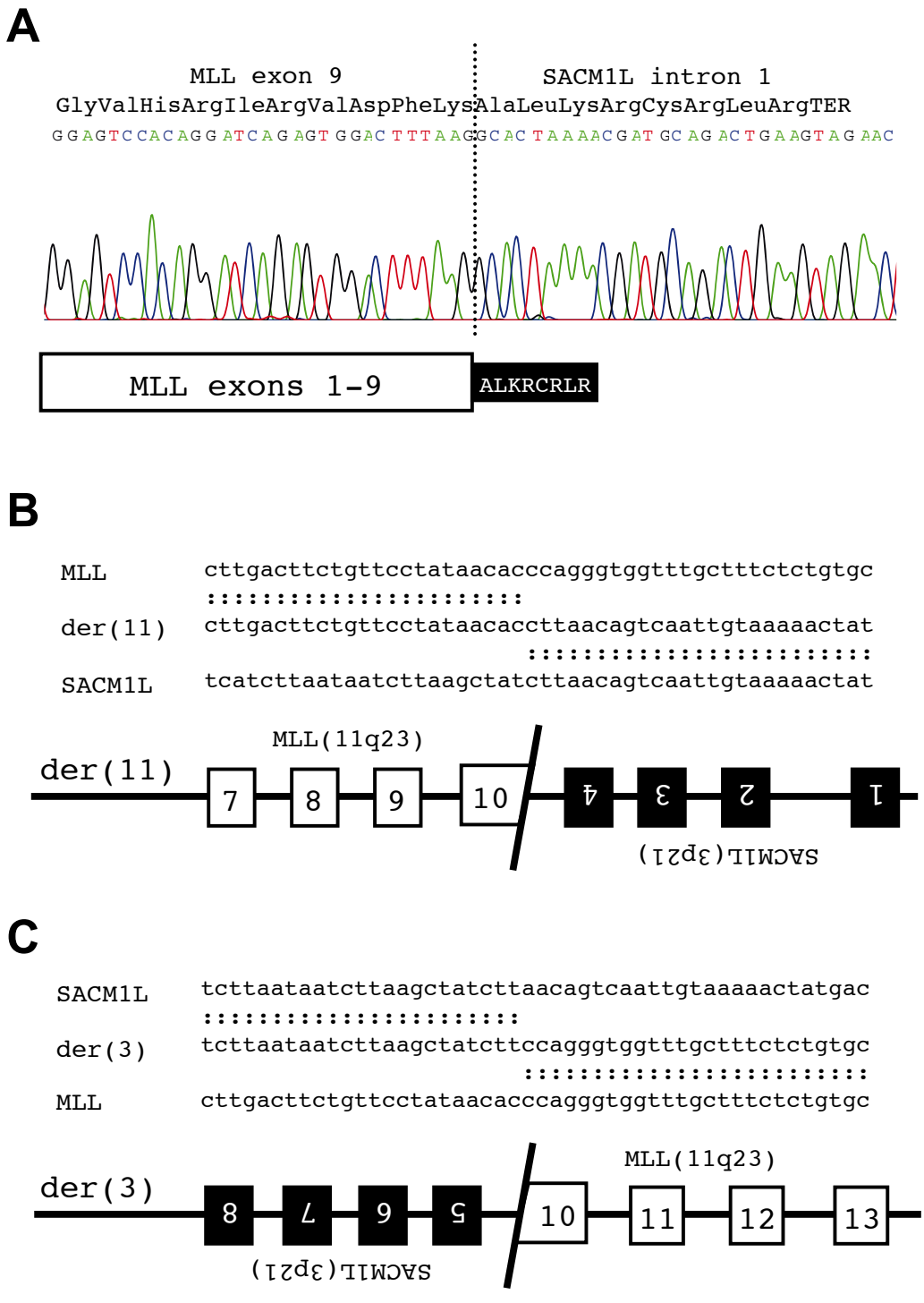


Fig. 3


A
MLL exon 9
SACM1L intron 1
GlyValHisArgIleArgValAspPheLysAlaLeuLysArgCysArgLeuArgTER
GGAGTCCACAGGATCAGAGTGGACTTTAAGGCACTAAAACGATGCAGACTGAAGTAGAAC
MLL exons 1-9
ALKRCRLR
B
MLL cttgacttctgttcctataacacccagggtggtttgctttctctgtgc
der(11) cttgacttctgttcctataacaccttaacagtcaattgtaaaaactat
SACM1L tcatcttaataatcttaagctatcttaacagtcaattgtaaaaactat
MLL(11q23)
der(11)
7
8
9
10
4
3
2
1
SACM1L(3p21)
C
SACM1L tcttaataatcttaagctatcttaacagtcaattgtaaaaactatgac
der(3) tcttaataatcttaagctatcttccagggtggtttgctttctctgtgc
MLL cttgacttctgttcctataacacccagggtggtttgctttctctgtgc
MLL(11q23)
der(3)
8
7
9
5
10
11
12
13
SACM1L(3p21)