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Unexpected appearance of *KMT2A::MLLT10* fusion transcript in acute myeloid leukemia with t(5;11)(q31;q23.3)

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Running title: *KMT2A::MLLT10* in AML with t(5;11)

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Abstract

As an uncommon but nonrandom translocation in acute myeloid leukemia (AML) t(5;11)(q31;q23) results in fusion between *KMT2A* at 11q23 and *ARHGAP26* at 5q31. The 5q31 region has another *KMT2A* partner, *AFF4*, which was identified in acute lymphoblastic leukemia harboring ins(5;11)(q31;q13q23). We report here a 65-year-old woman with AML M5b. G-banding and spectral karyotyping demonstrated 46,XX,t(5;11)(q31;q23.3). Fluorescence *in situ* hybridization revealed not only separated 5' and 3' *KMT2A* signals but a faint 5' *KMT2A* signal. Reverse transcription polymerase chain reaction (RT–PCR), using a *KMT2A* sense primer and *ARHGAP26* antisense primer, detected no band whereas RT–PCR with a *AFF4* antisense primer revealed an amplified band. However, sequence analysis unexpectedly disclosed that *KMT2A* exon 6 was connected with *MLLT10* exons 15 to 18. This may be due to cross-hybridization between *MLLT10* exon 18 and *AFF4* antisense primer derived from *AFF4* exon 10 since both exons had eight identical bases (AAGCAGCT). The *MLLT10* gene is located at 10p12.31; a faint 5' *KMT2A* signal was probably present at this locus. These findings indicate that in AML the 5' *KMT2A* fragment containing exons 1 to 6 may be cryptically inserted into *MLLT10* intron 14 when a reciprocal translocation t(5;11)(q31;q23.3) involving *KMT2A* occurred.

***Keywords*:** acute myeloid leukemia, *AFF4*, cryptic insertion, *KMT2A*, *MLLT10*, t(5;11)(q31;q23.3)

Introduction

The *KMT2A* gene (also known as *MLL*) located at 11q23.3 is involved in the leukemogenesis of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) by forming fusion genes with over 90 partners through chromosomal reciprocal translocations and inversions [1, 2]. Of these, the t(5;11)(q31;q23) is an uncommon but nonrandom translocation in AML, particularly for the M5 subtype of AML according to the French–American–British classification system [2, 3]. The fusion gene was generated between *KMT2A* at 11q23 and *ARHGAP26* (alias *GRAF*) at 5q31 in two cases of AML with t(5;11)(q31;q23) and a case of AML with ins(5;11)(q31;q23q23) [4-6]. In addition, the 5q31 region has another *KMT2A* partner, *AFF4* (alias *AF5q31*), which was initially identified in an infant with ALL harboring ins(5;11)(q31;q13q23) [7]. The resultant *KMT2A::AFF4* fusion transcript was also detected in two other infants with ALL showing complex karyotypes [8, 9]. Since *KMT2A* and *AFF4* are transcribed in opposite directions, a simple balanced translocation, t(5;11)(q31;q23), cannot produce an in-frame *KMT2A::AFF4* fusion gene.

We describe here an uncommon case of AML with t(5;11)(q31;q23.3) and *KMT2A* rearrangement. Unexpectedly, instead of *ARHGAP26* or *AFF4* at 5q31, this translocation resulted in a fusion gene between *KMT2A* and *MLLT10*. The results suggest that the 5' portion of *KMT2A* was cryptically inserted into *MLLT10* at 10p12 when a reciprocal translocation of t(5;11)(q31;q23.3) involving *KMT2A* occurred.

Materials and methods

Case report

A 65-year-old female was transferred to our hospital to undergo salvage therapy and stem cell transplantation for relapsed AML. At initial diagnosis in another hospital, peripher-

al blood counts were: hemoglobin 62 g/L, platelets 170 × 10^9/L, and white blood cells (WBC) 16.03 × 10^9/L with 56% promonocytes. The patient's bone marrow showed infiltration of 78.4% promonocytes, indicating a diagnosis of *de novo* AML M5b since the patient had no history of chemotherapy or radiotherapy for malignancies. She went into complete remission after induction treatment with idarubicin and cytarabine. However, the disease relapsed after three courses of consolidation therapy.

On admission, the patient's hypercellular bone marrow was infiltrated with 63.6% promonocytes and 26.8% monocytes. Large-sized promonocytes showed fine nuclear chromatin and a pale cytoplasm (Fig. 1A). Flow cytometric analysis demonstrated that gated cells (82.3% of bone marrow cells) by CD45/side scatter gating were positive for CD33 (99.7%), CD38 (99.8%), CD41 (28.0%), CD56 (71.8%), and HLA-DR (99.9%). However, gated cells were negative for CD13 (10.1%), CD14 (18.8%), and myeloperoxidase (0.8%). Molecular screening showed that cells were negative for tyrosine kinase domain mutations and internal tandem duplication of *FLT3*. A diagnosis of AML M5b at first relapse was made.

The patient received salvage therapy using a high-dose cytarabine regimen, but the WBC re-increased to 11.5 × 10^9/L with 51% promonocytes. Subsequently, she received further treatment with gemtuzumab ozogamicin with no noticeable effect. Seven months after the initial diagnosis, the patient died due to disease progression.

Cytogenetic analyses

Chromosome analyses were carried out using a G-banding method on short-term cultured cells from the bone marrow. Karyotypes were described in line with the International System for Human Cytogenetic Nomenclature (2020). Spectral karyotyping (SKY) using Sky Paint Probes (Applied Spectral Imaging, Carlsbad, CA, USA) was performed on meta-

phase cells. Fluorescence *in situ* hybridization (FISH) analyses with a Vysis LSI MLL Dual Color, Break Apart Rearrangement Probe Kit (Abbott Molecular, Abbott Park, IL, USA) were also performed on interphase nuclei and metaphase cells.

Reverse transcription polymerase chain reaction analyses

On admission, total RNA was extracted from bone marrow mononuclear cells using an RNeasy Mini Kit (Qiagen, Hilden, Germany). Total RNA was transcribed into cDNA using a SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). For detection of the *KMT2A::AFF4* chimeric transcript, a sense primer, KMT-e6 (from *KMT2A* exon 6, 5'-ATGCCTTCCAAAGCCTACCT-3', cDNA bases 3619–3638 according to NM_005933.4 of a NCBI reference sequence), and an antisense primer, AFF-e10 (from *AFF4* exon 10, 5'-AGTCAGATCCAGAGCTGCTTTC-3', cDNA bases 1683–1704 according to NM_014423.4) were designed. For detection of the *KMT2A::MLLT10* chimeric transcript, another sense primer KMT-e5 (from *KMT2A* exon 5, 5'-TCACTAGAAACAAGGCACCCC-3', cDNA bases 3425-3445) and an antisense primer MLL-e15 (from *MLLT10* exon 15, 5'-AGAGCGCTCCTACTTGTTGCA-3', cDNA bases 2148-2168 according to NM_004641.4) were used.

Reverse transcription polymerase chain reaction (RT–PCR) was performed using an AmpliTaq GOLD 360 Master Mix (Applied Biosystems, Foster City, CA, USA). The reaction mixture was denatured at 95°C for 10 min. Subsequently, 40 cycles of amplification were carried out: denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 60 sec. Finally, an extension at 72°C for 10 min was added. For amplification of the *KMT2A::AFF4* fusion, a nested PCR using the same primers under the same conditions was performed. The amplified DNA was electrophoresed on a 2.0% agarose gel along with a 100-bp DNA marker. Nucleotide sequence analysis was done using a BigDye Terminator

v3.1 Cycle Sequencing Kit (Applied Biosystems).

Results

The G-banded karyotypes of bone marrow cells at initial diagnosis and on admission were 46,XX,t(5;11)(q31;q23.3)[14]/46,XX[6] and 46,XX,t(5;11)(q31;q23.3)[2]/47,sl,+18[10]/48,sl,+18,+18[1]/46,XX[1], respectively (Fig. 1B). SKY revealed both the der(5)t(5;11)(q31;q23.3) and the der(11)t(5;11)(q31;q23.3); both chromosomes 10 showed no apparent abnormality (Fig. 1C). FISH using metaphase cells revealed a 5' *KMT2A* signal on der(11)t(5;11) and a 3' *KMT2A* signal on der(5)t(5;11), indicating *KMT2A* rearrangement. Furthermore, a faint 5' *KMT2A* signal was detected on another chromosome (Fig. 1D). FISH using interphase nuclei also showed a faint 5' *KMT2A* signal in addition to 5' *KMT2A*, 3' *KMT2A*, and 5'/3' *KMT2A* fusion signals (Fig. 1E).

To search for the *KMT2A* partner gene, we first carried out RT–PCR for the *KMT2A::ARHGAP26* fusion transcript as described previously [5], since the diagnosis of the present case was AML M5b but not ALL. However, no fusion transcript was detected (data not shown). Next, we performed RT–PCR to detect a *KMT2A::AFF4* fusion transcript. First PCR could not amplify a product, but nested PCR with the same primers yielded a clear amplified band (Fig. 2A). However, unexpectedly, sequence analysis of the amplified product disclosed that *KMT2A* exon 6 was connected to *MLLT10* exons 15 to 18, which was further connected with an *AFF4* antisense primer derived from *AFF4* exon 10 (Fig. 2B). Compared with sequences of *MLLT10* exon 18 and *AFF4* exon 10, eight bases (AAGCAGCT) were identical between them (Fig. 2C). Due to this similarity, nested PCR with primers for *KMT2A* and *AFF4* probably amplified a *KMT2A::MLLT10* fusion transcript by cross-hybridization. We then performed RT–PCR to confirm *KMT2A::MLLT10* fusion transcripts. As expected, an amplified band of 363 bp was identified (Fig. 2D). Sequence analy-

sis confirmed that *KMT2A* exon 6 and *MLLT10* exon 15 were connected in-frame (Fig. 2E, F). This *MLLT10* fusion point corresponds to AF10 1931 (GenBank accession number U13948) [10].

Discussion

We detected *KMT2A::MLLT10* chimeric transcripts in a patient with AML M5b. G-banding and SKY demonstrated only a reciprocal balanced translocation t(5;11)(q31;q23.3). FISH revealed split signals of 5' *KMT2A* and 3' *KMT2A*, suggesting that *KMT2A* was fused to its partner gene at 5q31. At this time, we could not recognize the significance of a faint 5' *KMT2A* signal. However, RT–PCR using an *KMT2A* sense primer and the antisense primers of possible 5q31 partner candidates, *ARHGAP26* and *AFF4*, accidentally detected a *KMT2A* exon 6-*MLLT10* exon 15 fusion transcript by cross-hybridization of *AFF4* antisense primer with *MLLT10*. Subsequently, we retrospectively investigated the results of FISH analyses. Considering that the *MLLT10* gene is located at 10p12.31, it is probable that a faint 5' *KMT2A* signal was present at this locus. Thus, the following mechanisms are suspected: Chromosomal breaks may have occurred at two locations in the 11q23 region. The genomic fragment corresponding to the faint 5' *KMT2A* signal was sandwiched between two breakpoints and contained exons 1 through 6 of *KMT2A*. This fragment was presumably inverted and inserted into *MLLT10* intron 14 at 10p12, resulting in the *KMT2A::MLLT10* fusion gene. In comparison, the 3' genomic region corresponding to the 3' *KMT2A* signal was translocated to chromosome 5. Namely, cryptic insertion of 5' *KMT2A* into *MLLT10* at 10p12 occurred simultaneously with a reciprocal t(5;11)(q31;q23.3) translocation involving *KMT2A* (Fig. 1F). These cytogenetic mechanisms are summarized in Figure 3.

The *MLLT10* gene (also called *AF10*) is the fourth most common *KMT2A* partner, and

the resultant *KMT2A::MLLT10* fusion gene is detected in adult AML, predominantly in M5, as well as infant and pediatric AML/ALL [2, 11]. Similar to *AFF4*, *MLLT10* is transcribed in an opposite direction (telomere to centromere) to *KMT2A*. Therefore, a simple t(10;11)(p12;q23) translocation cannot produce an in-frame *KMT2A::MLLT10* fusion gene. Various complex 10;11 rearrangements have been reported: three or more chromosome breaks and an inversion of one of the two genes are required [11, 12]. In the present case, it is probable that two breaks in the 11q23 region and one break in the 10p12 occurred. Recently, Peterson et al. summarized the cytogenetic results of 38 cases with acute leukemias harboring *KMT2A::MLLT10* fusions [12]. Of these, 10 cases (26%) showed either normal or abnormal karyotypes that did not involve chromosomes 10 or 11, suggesting cryptic *KMT2A::MLLT10* fusions located on 10p or 11q. Thus, the cytogenetic abnormalities leading to *KMT2A::MLLT10* fusion are various and complex, and, as observed in the present case, a combination of RT–PCR, G-banding, SKY, and FISH is essential to characterize such abnormalities [12].

Chromosomal 10;11 rearrangements resulting in *KMT2A::MLLT10* fusion are classified into four different types: the 11q segment containing 5' *KMT2A* is inverted and followed by translocation (type 1) or insertion (type 2) to 10p12. In comparison, an inversion of the 10p segment containing 3' *MLLT10* is accompanied by translocation (type 3) or insertion (type 4) to 11q23 [11]. The mechanism of the present case showed a type 2 pattern. Several cases of AML with an insertion of 5' *KMT2A* into *MLLT10* at 10p12 have been reported [13-16]. Morerio et al. reported a similar case of AML M5 with the 10;11 rearrangement involving a third chromosome: t(7;11;10)(q21;q21;p15) [13]. FISH revealed that the 5' region of *KMT2A* was inserted at 10p whereas the 3' region of *KMT2A* moved to a rearranged chromosome 7. However, in contrast to the present case, the inserted region was relatively large (11q14.2 to 11q23) and no *KMT2A* signal was found on the rearranged chromosome 11. To our

knowledge, the simultaneous occurrence of the cryptic insertion of 5' *KMT2A* into 10p and a reciprocal *KMT2A* translocation involving a third chromosome has not been described to date.

The structures of *KMT2A::MLLT10* chimeric genes seem to be various in AML/ALL with chromosomal 10;11 rearrangements. The *KMT2A* fusion points were shown to be concentrated in exons 9 to 11 [2, 17], all of which were included in the major breakpoint cluster region (exons 8 to 14) [18]. The *KMT2A* fusion point of the present case, exon 6, was located at the 5' side of the cluster region. In comparison, the *MLLT10* fusion points are scattered throughout the full length and extends from the 3' region of the zinc-finger domain to the 5' region of the leucine zipper domain [10, 19]. In the present case, the *MLLT10* fusion point, exon 15, is situated at the 5' region of the leucine zipper domain. It is currently unknown whether the forms of *KMT2A::MLLT10* fusion are associated with 10;11 rearrangement mechanisms or disease phenotypes [19].

In the present case, cryptic insertion of *KMT2A* into *MLLT10* could generate the *KMT2A::MLLT10* fusion but not the reciprocal *MLLT10::KMT2A*. Instead, it is possible that t(5;11)(q31;q23.3) formed a fusion between an unknown translocation partner gene (TPG) at 5q31 and 3' *KMT2A* (exons 7 to 36) on the der(5)t(5;11), since 3' *KMT2A* signal was translocated to der(5)t(5;11). Generally, *KMT2A*::TPG is transcribed in all *KMT2A*-rearranged leukemia cases, and its product is thought to be the major contributor to leukemogenesis [20], whereas a reciprocal TPG::*KMT2A* fusion seems to have a limited role. With regard to 5q31 partners, the reciprocal *ARHGAP26::KMT2A* and *AFF4::KMT2A* fusion transcripts were not expressed in AML with *KMT2A::ARHGAP26* and ALL with *KMT2A::AFF4*, respectively [4, 5, 7]. Exceptionally, in t(4;11)(q21;q23) leukemia cases, the reciprocal *AFF1::KMT2A* fusion gene is often expressed and its product exhibits oncogenic properties in mouse models [21]. Similarly, there might be a possibility that a TPG::*KMT2A* fusion gene on the

der(5)t(5;11) play some pathological role in the leukemogenesis as an additional genetic change.

In this study, we detected a *KMT2A::MLLT10* fusion transcript by chance in a case of relapsed AML. As described above, *KMT2A::MLLT10* fusions are usually generated by complex chromosomal aberrations requiring three or more breaks, and their frequency is relatively high. Thus, as observed in the present case, when *KMT2A* rearrangements are detected but the partner gene is unknown, and when *KMT2A* rearrangements by FISH are not the typical pattern, examining the expression of a *KMT2A::MLLT10* fusion transcript is recommended.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

[1] Marschalek R. Mechanisms of leukemogenesis by MLL fusion proteins. Br J Haematol 2011;152:141-154.

[2] Meyer C, Bumeister T, Gröger D, Tsaur G, Fechina L, Renneville A, et al. The *MLL* recombinome of acute leukemiaös in 2017. Leukemia 2018;32:273-284.

[3] Mitelman F, Johansson B, Mertens F (Eds.). Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (2022). https://mitelmandatabase.isb-cgc.org. accessed July 15, 2022.

[4] Borkhardt A, Bojesen S, Haas OA, Fuchs U, Bartelheimer D, Loncarevic IF, et al. The human *GRAF* gene is fused to *MLL* in a unique t(5;11)(q31;q23) and both alleles are disrupted in three cases of myelodysplastic syndrome/acute myeloid leukemia with a

deletion 5q. Proc Natl Acad Sci USA 2000;97:9168-9173.

[5] Panagopoulos I, Kitagawa A, Isaksson M, Mörse H, Mitelman F, Johansson B. *MLL/GRAF* fusion in an infant acute monocytic leukemia (AML M5b) with a cytogenetically cryptic ins(5;11)(q31;q23q23). Genes Chromosomes Cancer 2004;41:400-404.

[6] Wilda M, Perez AV, Bruch J, Woessnann W, Metzler M, Fuchs U, et al. Use of *MLL/GRAF* fusion mRNA for measurement of minimal residual disease during chemotherapy in an infant with acute monoblastic leukemia (AML-M5). Genes Chromosomes Cancer 2005;43:424-426.

[7] Taki T, Kano H, Taniwaki M, Sako M, Yanagisawa M, Hayashi Y. *AF5q31*, a newly identified *AF4*-related gene, is fused to *MLL* in infant acute lymphoblastic leukemia with ins(5;11)(q31;q13q23). Proc Natl Acad Sci USA 1999;96:14535-14540.

[8] Imamura T, Morimoto A, Ikushima S, Kakazu N, Hada S, Tabata Y, et al. A novel infant acute lymphoblastic leukemia cell line with *MLL-AF5q31* fusion transcript. Leukemia 2002;16:2302-2308.

[9] Deveney R, Chervinsky DS, Jani-Sait SN, Grossi M, Aplan PD. Insertion of *MLL* sequences into chromosome band 5q31 results in an *MLL-AF5q31* fusion and is a rare but recurrent abnormality associated with infant leukemia. Genes Chromosomes Cancer 2003;37:326-331.

[10] Chaplin T, Bernard O, Beverloo HB, Saha V, Hagemeijer A, Berger R, et al. The t(10;11) translocation in acute myeloid leukemia (M5) consistently fuses the leucine zipper motif of AF10 onto the HRX gene. Blood 1995;86:2073-2076.

[11] Van Limbergen H, Poppe B, Janssens A, De Bock R, De Paepe A, Noens L, et al. Molecular cytogenetic analysis of 10;11 rearrangements in acute myeloid leukemia. Leukemia 2002;16:344-351.

[12] Peterson JF, Sukov WR, Pitel BA, Smoley SA, Pearce KE, Meyer RG, et al. Acute leu-

kemias harboring *KMT2A/MLLT10* fusion: a 10-year experience from a single genomics laboratory. Genes Chromosomes Cancer 2019;58:567-577.

[13] Morerio C, Rapella A, Rosanda C, Lanino E, Lo Nigro L, Di Cataldo A, et al. *MLL-MLLT10* fusion in acute monoblastic leukemia: variant complex rearrangements and 11q proximal heterogeneity. Cancer Genet Cytogenet 2004;152:108-112.

[14] Christiansen L, Allen RA, Dunn ST, Wolff DJ. A case of infantile acute myelogenous leukemia with *MLL-MLLT10* fusion caused by insertion of 11q into 10p. Cancer Genet Cytogenet 2005;159:181-183.

[15] Jarosova M, Takacova S, Holzerova M, Priwitzerova M, Divoka M, Lakoma I, et al. Cryptic *MLL-AF10* fusion caused by insertion of duplicated 5' part of *MLL* into 10p12 in acute leukemia: a case report. Cancer Genet Cytogenet 2005;162:179-182.

[16] Matsuda K, Hidaka E, Ishida F, Yamauchi K, Makishima H, Ito T, et al. A case of acute myelogenous leukemia with *MLL-AF10* fusion caused by insertion of 5' *MLL* into 10p12, with concurrent 3' *MLL* deletion. Cancer Genet Cytogenet 2006;171:24-30.

[17] Zerkalenkova E, Lebedeva S, Kazakova A, Tsaur G, Starichkova Y, Timofeeva N, et al. Acute myeloid leukemia with t(10;11)(p11-12;q23.3): Results of Russian pediatric AML registration study. Int J Lab Hematol 2019;41:287-292.

[18] Meyer C, Lopes BA, Caye-Eude A, Cavé H, Arfeuille C, Cuccuini W, et al. Human *MLL/KMT2A* gene exhibits a second breakpoint cluster region for recurrent MLL-USP2 fusions. Leukemia 2019;33:2306-2310.

[19] Klaus M, Schnittger S, Haferlach T, Dreyling M, Hiddemann W, Schoch C. Cytogenetics, fluorescence in situ hybridization, and reverse transcriptase polymerase chain reaction are necessary to clarify the various mechanisms leading to an *MLL-AF10* fusion in acute myelocytic leukemia with 10;11 rearrangement. Cancer Genet Cytogenet 2003;144:36-43.

[20] Yokoyama A. Transcriptional activation by MLL fusion proteins in leukemogenesis. Exp Hematol 2017;46:21-30.

[21] Bursen A, Schwabe K, Rüster B, Henschler R, Ruthardt M, Dingermann T, et al. The AF4·MLL fusion protein is capable of inducing ALL in mice without requirement of MLL·AF4. Blood 2010;115:3570-3579.

Figure legends

Fig. 1. Cytogenetic and morphological findings of leukemic cells in bone marrow.

(A) Bone marrow smear shows large-sized promonocytes with fine nuclear chromatin and pale cytoplasm (×1000, May–Grünwald–Giemsa stain).

(B) The karyotype by G-banding is 46,XX,t(5;11)(q31;q23.3). Two arrows indicate der(5)t(5;11) and der(11)t(5;11).

(C) Spectral karyotyping (SKY) of metaphase spreads (right, SKY; left, reverse DAPI). Chromosomes 5, 11, and 10 are presented. Both der(5)t(5;11)(q31;q23.3) and der(11)t(5;11)(q31;q23.3) were found. No apparent abnormality was found on chromosomes 10. DAPI, 4,6-diamidino-2-phenylindole

(D) Fluorescence *in situ* hybridization (FISH) on metaphase cells using a Vysis LSI MLL Dual Color, Break Apart Rearrangement Probe. The arrows point to 1) a faint 5' *KMT2A* signal (green) on chromosome 10, 2) a normal 5' *KMT2A*/3' *KMT2A* fusion signal (yellow) on a normal chromosome 11, 3) a 5' *KMT2A* signal (green) on der(11)t(5;11), and 4) a 3' *KMT2A* signal (red) on der(5)t(5;11).

(E) FISH on interphase nuclei using the same probe. Red, green, and yellow signals were found on an interphase nucleus. In addition, a faint green signal (arrow) was detected.

(F) Schematic presentation of the *KMT2A* gene and putative breakpoints in the 11q23 region. The position of a Vysis LSI MLL probe (5' *KMT2A* and 3' *KMT2A* probes) covering the *KMT2A* gene on a normal chromosome 11 is presented. Two vertical arrows indicate assumed breakpoints; that is, the 5' side of *KMT2A* exon 1 and 3' side of *KMT2A* exon 6. An 11q23 fragment containing *KMT2A* exons 1 to 6 may be inserted into *MLLT10* at 10p12, resulting in der(10)ins(10)(p12q23q23).

Fig. 2. RT–PCR and sequence analyses of leukemic cells in the bone marrow.

(A) Expression of *KMT2A::MLLT10::AFF4* chimeric transcripts by reverse transcription polymerase chain reaction (RT–PCR) using KMT-e6 and AFF-e10 primers. Lane M, 100-bp DNA size markers; lane 1, leukemic cells from the patient. An amplified band of 430 bp was detected.

(B) A *KMT2A::MLLT10::AFF4* fusion transcript is shown schematically. White, gray, and small white boxes correspond to *KMT2A*, *MLLT10*, and *AFF4* exons, respectively. Horizontal arrows indicate the positions of KMT-e6 and AFF-e10 primers. The numbers of nucleotides and exons originate from National Center for Biotechnology Information (NCBI) reference sequences.

(C) Nucleotide sequences of the PCR product, *MLLT10* exon 18, *AFF4* exon 10, and *AFF4* antisense primer (AFF-e10) were compared. Eight nucleotides (AAGCAGCT) were identical between *MLLT10* exon 18 and *AFF4* exon 10. Thus, the *AFF4* antisense primer probably cross-hybridized with *MLLT10* exon 18.

(D) Expression of *KMT2A::MLLT10* chimeric transcripts by RT–PCR using KMT-e5 and MLL-e15 primers. Lane M, size markers (100-bp DNA ladder); lane 1, leukemic cells from the patient; and lane 2, negative control (normal bone marrow mononuclear cells). An amplified band of 363 bp was detected in the patient's cells.

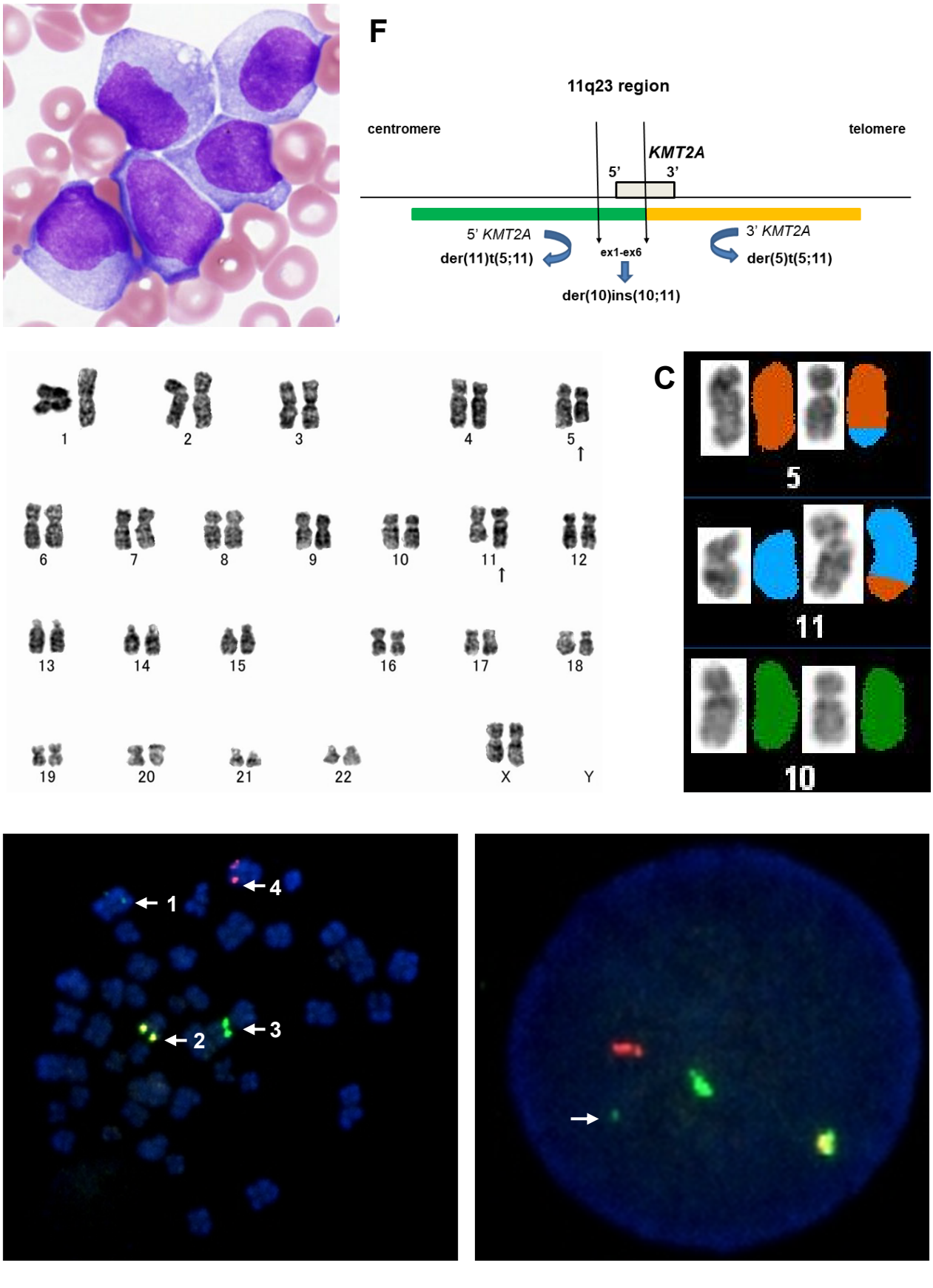
(E) A *KMT2A::MLLT10* fusion transcript is shown schematically. White and gray boxes correspond to *KMT2A* and *MLLT10* exons, respectively. Horizontal arrows indicate the positions of KMT-e5 and MLL-e15 primers. The number of nucleotides and exons originate from the NCBI reference sequences.

(F) Amino acid and nucleotide sequences around the breakpoint of the *KMT2A::MLLT10* chimeric transcript. *KMT2A* exon 6 and *MLLT10* exon 15 are connected in-frame. The breakpoint is indicated by a vertical arrow.

Fig. 3. Schematic presentation of the partial karyotype of chromosomes 5, 10, and 11 including normal chromosomes (left) and derivative chromosomes (right).

Idiograms of G-banding patterns for t(5;11)(q31;q23.3) and ins(10;11)(p12q23q23) at 300-band levels are shown. Chromosomal breakpoints are indicated by horizontal arrows. The location of the related genes (*AFF4*, *ARHGAP26*, *KMT2A*, and *MLLT10*) are shown on the normal chromosomes. 5' *KMT2A* (green) and 3' *KMT2A* (red) signals on normal and derivative chromosomes are also shown.

Fig. 1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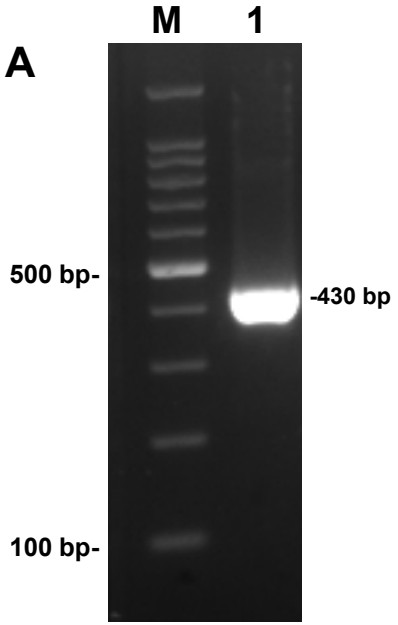
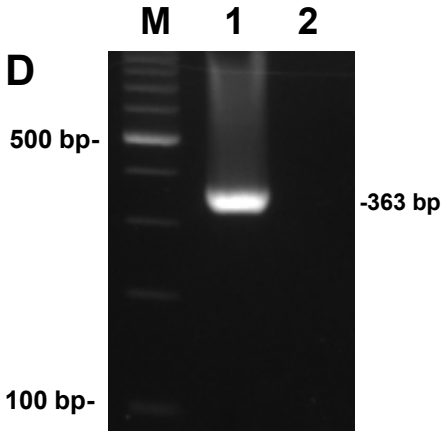
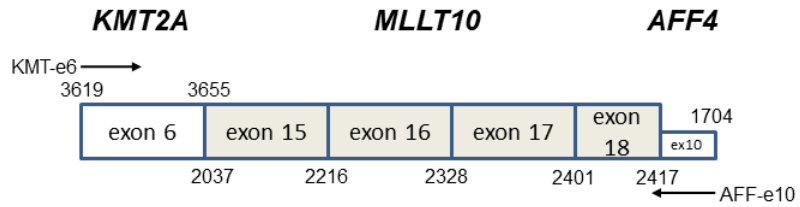
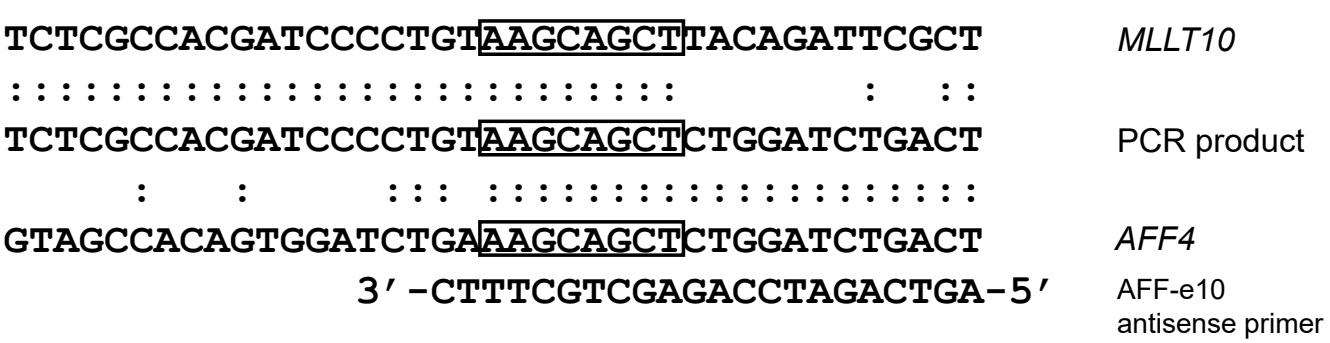
17
18
19
20
21
22
X
Y
C
5
11
10
D
1
2
3
4
E

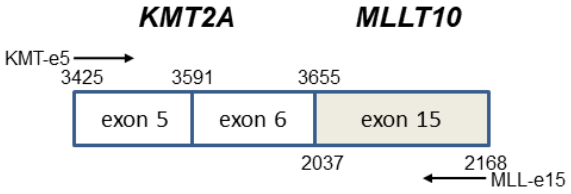
Fig. 2


A
M
1
500 bp-
100 bp-
-430 bp


D
M
1
2
500 bp-
100 bp-
-363 bp


B
KMT2A
MLLT10
AFF4
KMT-e6
3619
3655
exon 6
exon 15
exon 16
exon 17
exon 18
1704
ex10
2037
2216
2328
2401
2417
AFF-e10


C
TCTCGCCACGATCCCCTGTAAGCAGCTTACAGATTCGCT
MLLT10
TCTCGCCACGATCCCCTGTAAGCAGCTCTGGATCTGACT
PCR product
GTAGCCACAGTGGATCTGAAAGCAGCTCTGGATCTGACT
AFF4
3′-CTTTCGTCGAGACCTAGACTGA-5′
AFF-e10
antisense primer


E
KMT2A
MLLT10
KMT-e5
3425
3591
3655
exon 5
exon 6
exon 15
2037
2168
MLL-e15

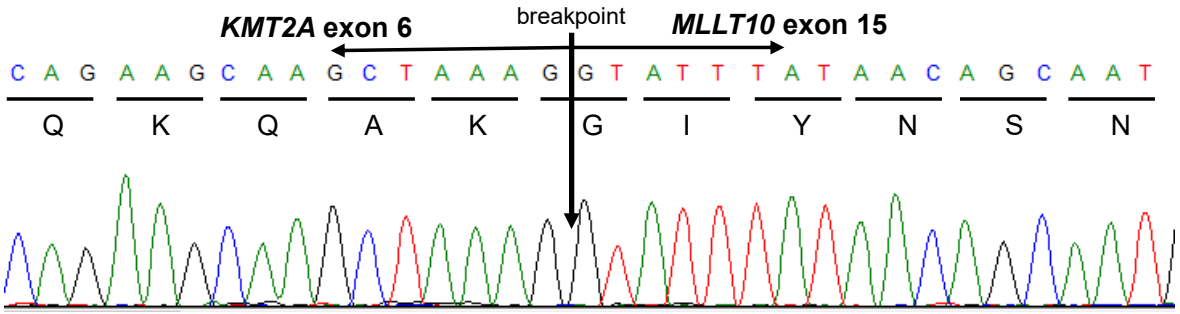
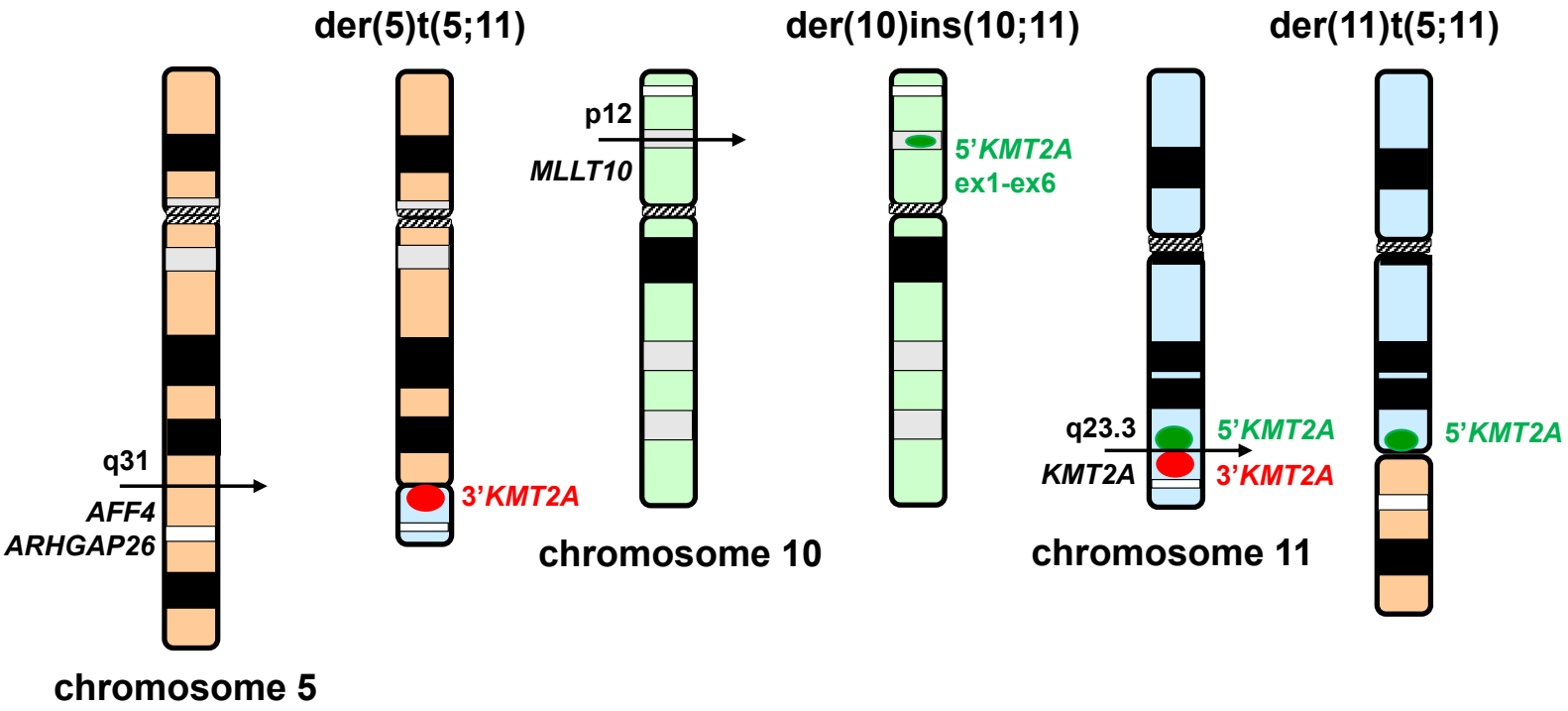

F
KMT2A exon 6
breakpoint
MLLT10 exon 15
C A G A A G C A A G C T A A A G G T A T T T A T A A C A G C A A T
Q K Q A K G I Y N S N

Fig. 3


der(5)t(5;11)
der(10)ins(10;11)
der(11)t(5;11)
q31
AFF4
ARHGAP26
3'KMT2A
p12
MLLT10
5'KMT2A
ex1-ex6
q23.3
KMT2A
5'KMT2A
3'KMT2A
5'KMT2A
chromosome 5
chromosome 10
chromosome 11