AF17q25, a Putative Septin Family Gene, Fuses the MLL Gene in Acute Myeloid Leukemia with t(11;17)(q23;q25)1

Tomohiko Taki, Hiroaki Ohnishi, Kenji Shinohara, Masahiro Sako, Fumio Bessho, Masayoshi Yanagisawa, and Yasuhisa Hayashi2

Department of Pediatrics, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113-8655 [T. T., K. O., M. Y., Y. H.]; Division of Hematology, Yamaguchi Prefecture Central Hospital, Hofs, Yamaguchi 747-8511 [K. S.]; and Department of Pediatrics, Osaka City General Hospital, Miyakojima-ku, Osaka 543-0021 [M. S.], Japan

Abstract

The t(11;17) has been described in patients with acute myeloid leukemia (AML), and the AF17 gene was previously cloned as a fusion partner of the MLL gene in t(11;17)(q23;q21)-AML. We analyzed one patient with de novo AML and one with therapy-related AML with t(11;17)(q23;q25) and identified the AF17q25 gene on chromosome 17q25, a putative septin family gene, fused with MLL. AF17q25 encoded at least three kinds of proteins [type I (568 a.a.), type II (594 a.a.), and type III (574 a.a.)] that contained two kinds of different amino acid sequences at the COOH terminus. The MLL-AF17q25 fusion transcript consisted of type I AF17q25 transcript. The AF17q25 protein is homologous to septin family proteins, including H5, NEDD5, CDC10, and hCDCrel, which is one of the fusion partners of MLL in t(11;22)(q23;q11)-AML. These results suggest that AF17q25 and hCDCrel might define a new septin family particularly involved in the pathogenesis of 11q23-associated leukemia.

Materials and Methods

Patients. Patient 1, a 24-year-old male, was initially diagnosed as AML (M2) with t(8;21)(q22;q22). He achieved a complete remission by chemotherapy with daunorubicin, cytarabine, and prednisone and received consolidation chemotherapy that included cytarabine. During remission, AML1-MTG8 chimeric mRNA was detected by RT-PCR. After 18 months, he developed AML (M4) with t(11;17)(q23;q25), without AML1-MTG8 chimeric mRNA. Although he received induction therapy with daunorubicin and cytarabine, he did not achieve a remission. Patient 2, a 4-month-old girl, was diagnosed as de novo AML (M5) with t(11;17)(q23;q25). She achieved a complete remission by chemotherapy and received a cord blood stem cell transplant 15 months after diagnosis. She has continued in complete remission for 5 years.

Southern Blot Analysis. High molecular weight DNA was extracted from bone marrow cells from patient 1 by proteinase K digestion and phenol/chloroform extraction (16). DNA (5 μg) was digested with appropriate restriction enzymes, subjected to electrophoresis on 0.7% agarose gels, transferred to charged nylon filters (Amershams Corp.), and hybridized with a cDNA probe labeled by the random hexamer method (16). A 0.9-kb BamHI fragment (designated probe x) derived from MLL cDNA was used as a probe (9).

Preparation of mRNA and cDNA Libraries. Poly(A)+ RNA from frozen cells derived from patient 1 was extracted with a Fast Track mRNA Isolation Kit (Invitrogen, San Diego, CA). Briefly, random hexanucleotide-primed synthesized cDNAs were ligated with EcoRI adapters and cloned into the EcoRI-digested Agt10 cloning vector (Promega, Madison, WI). After packaging with a commercial packaging kit (Epitcine Technologies, Madison, WI), phage plaques were screened with probes labeled using a random primer synthesis kit (Strategene, La Jolla, CA). The probes used for screening were probe x for the patient cDNA library and a 325-bp AF17q25 cDNA probe derived from a MLL-AF17q25 chimeric clone for the human placenta cDNA library (Clontech, Palo Alto, CA).

RT-PCR. Total cellular RNA was extracted from bone marrow cells of the patients by the acid guaniidine isothiocyanate-phenol-chloroform method (17). Total RNA (4 μg) was reverse transcribed to cDNA in a total volume of 33 μl with random hexamers and 20 units of reverse transcriptase (AMV; Boehringer Mannheim, Mannheim, Germany). cDNA (1 μl) was amplified by PCR in a total volume of 50 μl with 50 mM KCl, 1.5 mM MgCl2, 10 mM Tris-HCl (pH 9.0 at room temperature), 25 pmol of each primer, 75 μM of each dNTP, and 2.5 μl of Taq polymerase (Ampli Taq Gold; Applied Biosystems, Urayasu, Japan). After 35 rounds of PCR (30 s at 94°C, 30 s at 55°C, and 1 min at 72°C), 5 μl of PCR product were electrophoresed in a 1% agarose gel. The primers used were: MLL-5S, 5'-TACTACAGGACCGCAAGAA-3' and MLL-8A, 5'-CTCCATCCTCCCAACATGTT-3'. 5'-TCAACAAGAACTGCTGG-3'; 17-5A, 5'-CAGAGCCTGATGAAACGAAC-3'; 17-11S, 5'-CGGATGCATTCGTGATTGCA-3'; 17-11S, 5'-CCGATGCATTCGTGATTGCA-3'; 17-11S, 5'-CGGATGCATTCGTGATTGCA-3'; 17-11S, 5'-CGGATGCATTCGTGATTGCA-3'; 17-11S, 5'-CGGATGCATTCGTGATTGCA-3'.

Nucleotide Sequencing. The PCR products were cloned into the TA cloning vector (Invitrogen). The nucleotide sequences of plasmid clones and PCR products were determined by the fluorescent method (Ref. 16; Dye Terminator Cycle Sequencing Kit; Applied Biosystems).

Northern Blot Analysis. Multiple human-tissue Northern blots (Clontech) were hybridized with a 32P-labeled probe. A 589-bp PCR product (nucleotides 637–1225) including the breakpoint of t(11;17) was used as a probe.

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2 To whom requests for reprints should be addressed, at Department of Pediatrics, Faculty of Medicine, University of Tokyo, 2-11-30 Yoyogi, Bunkyo-ku, Tokyo 113-8655, Japan. Phone: 81-3-3815-5411, ext. 33452; Fax: 81-3-3816-4108; E-mail: hayashi@ped.h.u-tokyo.ac.jp.

3 The abbreviations used are: AML, acute myeloid leukemia; RT-PCR, reverse transcription-PCR.

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Results

Rearrangement of MLL and Noninvolvement of AF17. Southern blot analysis of DNA prepared from the leukemic cells of patient 1 using probe x revealed a chromosomal breakpoint within the breakpoint cluster region of the MLL gene at 11q23 (Fig. 1) that spans exons 5–11 in the MLL locus. However, no PCR products were amplified from the cDNA prepared from these cells when the MLL-AF17-specific primer pair was used. We inferred that the MLL in this patient is fused to a novel partner gene.

Isolation of the MLL Fusion cDNAs from t(11;17)(q23;q25). To isolate fusion transcripts of MLL, we prepared a cDNA library from the mRNA of leukemic cells from patient 1. Four cDNA clones were isolated by screening with probe x, and one of them (clone 17-2) was found to represent a fusion transcript of MLL. Clone 17-2, 766-bp in size, contained a 441-bp sequence corresponding to exons 4c to 5 in the mRNA of leukemic cells from patient 1. Four cDNA clones were isolated by screening with probe x, and one of them (clone 17-2) was found to represent a fusion transcript of MLL. Clone 17-2, 766-bp in size, contained a 441-bp sequence corresponding to exons 4c to 5 in the MLL gene at the 5’ region, and the remaining 325-bp sequence did not match the MLL gene or the partner genes of MLL that were previously cloned (Fig. 2A).

Isolation of the AF17q25 Gene That Encodes a Putative Septin Family Protein. The 325-bp sequence identified in the chimeric clone was used as a probe to screen a human placenta cDNA library. We isolated two kinds of clones (Fig. 2B). Clones 4-1 and 5-1 contained sequences of 4472 and 3044 nucleotides, respectively, with an open reading frame encoding a protein of 568 amino acids with a predicted molecular weight of M, 53,711 (Fig. 2C). Sequence comparisons of the predicted AF17q25 protein using the BLAST file showed significant similarity to the septin family proteins (18) (Fig. 3). One of the fusion partners of MLL, hCDCrel (19), which belongs to the septin family, is also homologous to AF17q25. Secondary-structure prediction using the MOTIF program on the web indicated that AF17q25 contains a guanosine 5’-triphosphate-binding region (residues 287–294, Fig. 2C).

Detection of the MLL-AF17q25 Fusion Transcripts in de novo and Therapy-related AML. Using a sense primer from MLL exon 5 (MLL-5S) and an antisense primer from AF17q25 (17-1A), we obtained PCR products of 209 bp from both patients (Fig. 4A). Reciprocal PCR products of AF17q25-MLL fusion transcripts were generated by RT-PCR using a sense primer from AF17q25 (17-1S) and an antisense primer from MLL exon 8 (MLL-8A) in both patients (Fig. 4A). However, sequence analysis revealed that their fusions, same position of AF17q25 (nucleotide 834) to exon 7 of MLL, were out of frame.

AF17q25 Encodes Three Kinds of Proteins That Contained Different Amino Acid Sequences at the COOH Terminus. We analyzed the AF17q25 sequence for expressed sequence tags using the BLAST file and found that some kinds of expressed sequence tags matched the AF17q25 sequence, but also found that AF17q25 seemed to have some kinds of splicing variants in the coding region. We performed RT-PCR analysis for EBV-induced B cell lines from healthy volunteers using primers 17-1S and 17-1A and detected only products of about 600 bp (Fig. 4A). Sequence analysis revealed that these products were derived from two kinds of AF17q25 transcripts: one product lacked an 1849-bp sequence of AF17q25 (nucleotides 2415–2463), and the other lacked an 1801-bp sequence (nucleotides 2463–2463; Figs. 2, B and C). The predicted amino acid sequences encoded by these two kinds of transcripts contained 594 (type II) and 579 (type III) amino acids, which are larger than the protein encoded by the longer transcript (type I) (Fig. 2C). The type II and type III proteins were completely different from the type I protein at the COOH terminus, 34 amino acids in the type I protein and 44 amino acids in types II and III proteins (Fig. 2C). Furthermore, we performed RT-PCR analysis for EBV-induced B cell lines using primers 17-1S and 17-5A, which could detect only type I AF17q25 transcripts, and detected a PCR product of an expected size (Fig. 4A), suggesting that at least three kinds of AF17q25 transcripts were expressed.

MLL-AF17q25 Fusion Transcripts Consist of Type I AF17q25 Transcripts. To study the expression of MLL-AF17q25 fusion transcripts in the patient’s leukemic cells, we performed RT-PCR analysis for RNA from the leukemic cells using primers MLL-5S and 17-5A or 17-11A. However, no PCR product was obtained from either patient, suggesting that the quality of mRNA from the patients’ leukemic cells was not good (data not shown). We next performed RT-PCR using primers 17-11S and 17-5A or 17-11A for detecting type I or types II/III AF17q25 transcripts from patients’ leukemic cells and detected about a 700-bp product only when primers 17-11S and 17-5A were used (Fig. 4A), suggesting that a MLL-AF17q25 fusion transcript is formed by type I AF17q25 in both patients.

Expression of the AF17q25 Gene in Normal Tissues. To study the expression of the AF17q25 gene, we performed Northern blot analysis on poly(A)-selected RNA from various human tissues. The expression of transcripts of about 4.0 kb was found in all tissues examined, and in some adult tissues (heart, placenta, liver, skeletal muscle), some additional, different-sized bands were observed (Fig. 4B), suggesting that there may be some kinds of alternatively spliced transcripts of AF17q25.

Discussion

The t(11;17) has been identified previously, and the AF17 gene was cloned as a fusion partner of the MLL gene in AML with t(11;17)(q23; q21) (10). However, cytogenetic analysis showed the presence of another breakpoint at 17q in AML with t(11;17)(q23;q25). In the present study, we isolated a novel chimeric transcript, MLL-AF17q25, in two patients with AML (FAB-M4 and M5) with t(11;17)(q23;q25). Recent molecular analysis has revealed more than two genes in the same chromosomal region as fusion partners of the MLL [i.e., the ENL, ELL/MEN (20, 21), and EEN (22) genes in 19p13.1–13.3 and ABI-I (16) and AF10 (11) genes in 10p11.2-12], suggesting that molecular analysis is essential for the precise localization of the chromosomal translocations.
Fig. 2. A, partial sequences of the junctions of the MLL-AF17q25 chimeric transcripts. Arrows, a fusion point. B, cloning of AF17q25 cDNA clones isolated from human placenta cDNA library. Types II and III were isolated from EBV-induced B cell line by RT-PCR using primers 17-11S and 17-11A. C, sequencing of the AF17q25 cDNA. *, a termination codon. Underlined residues 287–294 indicate a potential guanosine 5'-triphosphate-binding site. Arrow, the breakpoint in both patients. Type II transcript lacks between arrowheads ② and ③. Type III transcript lacks between arrowheads ① and ③.
AF17q25 is homologous to septin family proteins. The septins are a family of nucleotide-binding proteins originally found in the yeast *Saccharomyces cerevisiae* as cell division cycle regulatory proteins (18). CDC3, CDC10, CDC11, and CDC12 in yeast; Pnut, Sep1, and Sep2 in *Drosophila*; and Nedd5, H5, and Diff6 in mouse have been identified as septin family proteins. Septins are associated with actin stress fibers in interphase cells, the cleavage furrow of dividing cells, and the bud neck of budding yeast. The function of the septins is considered to be related to the organization of specialized domains within the cells. Recently, the hCDCrel gene, a member of the septin family, was also identified as one of the fusion partners of the MLL (19). Sequence analysis revealed that the AF17q25 protein is homologous to hCDCrel (46% identity, 84% similarity; Fig. 4).

MLL is considered to be a transcriptional maintenance factor and some of its downstream targets are Hox genes (23). At present, more than 15 partner genes for *MLL* have been cloned from leukemia cells with various types of reciprocal 11q23 translocations, including t(4; 11), t(9;11), and t(11;19). The functions of some genes have been revealed, including those for a Ras-binding protein (AF6; Ref. 24), a RNA polymerase II elongation factor (ELL/MEN; Ref. 25), a transcriptional coactivator/histone acetyltransferase (CBP and p300; Refs. 14 and 15), and ABL and eps8-binding protein ABI-1 (16). The functions of the fusion transcripts have remained unknown. Two studies showed that chimeric mice carrying the mouse *Mll-AF9* fusion gene developed AML (26) and that hematopoietic progenitor cells transduced with MLL-ENL induced AML in vitro (27), respectively. Rearrangements of the *MLL* gene found in acute lymphoblastic leukemia, AML, and myelodysplastic syndrome suggest that this gene fused to various partner genes plays a causative role in the dysregulation of differentiation along both lymphoid and myeloid pathways. Further functional analyses of the MLL-AF17q25, as well as MLL-hCDCrel, may provide new insights into leukemogenesis in patients involving 11q23 translocations.

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Note Added in Proof

After completion of this manuscript, Osaka et al. reported the cloning of the MSF gene, which fuses to the *MLL* gene in therapy-related AML with t(11;17)(q23;q25) [Osaka, M., Rowley, J. D., and Zeleznik-Le, N. J. MSF (MLL septin-like fusion), a fusion partner gene of *MLL*, in a therapy-related acute myeloid leukemia with a t(11;17)(q23;q25). Proc. Natl. Acad. Sci. USA, 96: 6428 – 6433, 1999]. The coding sequence of AF17q25 type I is the same as that of MSF, except for one nucleotide (nucleotide number 1192, codon 127).

References

MLL-AF1q25 FUSION IN t(11;17)-ALL


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