Clathrin Assembly Lymphoid Myeloid Leukemia-AF10-positive Acute Leukemias: A Report of 2 Cases with a Review of the Literature

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INTRODUCTION

The translocation t(10;11)(p13;q14q21) has been found to be recurrent in acute lymphoblastic and myeloid leukemias, and results in the fusion of the clathrin assembly lymphoid myeloid leukemia (CALM) gene with the AF10 gene; these genes are present on chromosomes 11 and 10, respectively. Because the CALM-AF10 rearrangement is a rare chromosomal abnormality, it is not included in routine molecular tests for acute leukemia. Here, we describe the cases of 2 patients with the CALM-AF10 fusion gene. The first patient (case 1) was diagnosed with T-cell ALL, and the second patient (case 2) was diagnosed with AML. Both patient samples showed expression of the homeobox A gene cluster and the histone methyltransferase hDOT1L, which suggests that they mediate leukemic transformation in CALM-AF10-positive and mixed-lineage leukemia-AF10-positive leukemias. Both patients achieved complete remission after induction chemotherapy. The first patient (case 1) relapsed after double-unit cord blood transplantation; there was no evidence of relapse in the second patient (case 2) after allogenic peripheral blood stem cell transplantation. Since CALM-AF10-positive leukemias have been shown to have poor prognosis with conventional therapy, molecular tests for CALM-AF10 rearrangement would be necessary to detect minimal residual disease during follow-up. (Korean J Lab Med 2010;30:117-21)

Key Words: t(10;11), CALM-AF10, HOXA gene, hDOT1L, Acute leukemia, Reverse transcriptase-PCR
are characterized by the following factors: young age of patients, mixed-lineage immunophenotype with coexpression of T-cell and myeloid antigens, extramedullary involvement, T-cell receptor γδ (TCRγδ) lineage, and poor clinical outcome [6, 7]. Recently, several genes of the homeobox A (HOXA) cluster, including HOXA5, HOXA9, and HOXA10, were shown to be upregulated in CALM-AF10-positive leukemic cells, with expression profiles similar to those of mixed-lineage leukemia (MLL)-AF10 fusion gene-positive leukemias [8]. Overexpression of HOXA5 through the methylation of lysine 79 of histone H3 (H3K79), mediated by human DOT1-like, histone H3 methyltransferase (hDOT1L) has been suggested as the key mechanism by which the CALM-AF10 fusion gene causes leukemogenesis [9]. Here, we report 2 cases of CALM-AF10-positive leukemias with a HOX A gene cluster/hDOT1L expression pattern. To our knowledge, only a single case of CALM-AF10-positive AML has previously been reported in Korea [10]. This is the first report of CALM-AF10-positive ALL in Korea.

CASE REPORTS

1. Case 1

An 18-yr-old woman was referred for further evaluation and treatment of a neck mass and cytopenia. A physical examination revealed a non-tender, fixed 5-cm mass on the left side of the neck. A complete blood cell count and peripheral blood smear showed white cell count (WBC) and platelet counts of $10.4 \times 10^9$ and $193 \times 10^9$, respectively, with 37% leukemic blasts; the hemoglobin level was 9.2 g/dL. The bone marrow was hypercellular, with diffuse infiltration of leukemic blasts (67%) (Fig. 1A). Immunophenotyping of bone marrow cells showed positive expression of cytCD3 (89%), CD7 (88%), CD13 (87%), CD33 (24%), CD34 (89%), and Tdt (89%); the cells were negative for myeloperoxidase. The patient was diagnosed with precursor T-cell ALL with aberrant expression of myeloid antigen. A cytogenetic analysis showed that the karyotype of the bone marrow cells was 46,XX,del(1)(p35),t(10;11)(p13;q21)[17]/46,XX[5]. The patient received induction and consolidation chemotherapy and achieved complete remission. After 2 additional cycles of consolidation chemotherapy, she underwent double-unit cord blood transplantation. Although the engraftment was successful, the patient relapsed after 4 months.

2. Case 2

A 34-yr-old man was admitted for further evaluation of pancytopenia. Initial laboratory tests showed a hemoglobin level of 10.8 g/dL, WBC count of $0.9 \times 10^9$, and platelet count of $85 \times 10^9$. A peripheral blood smear revealed that

Fig. 1. Bone marrow aspiration shows several lymphoblasts in the first patient (case 1) (A) and myeloblasts in the second patient (case 2) (B) characterized by medium-sized nuclei and prominent nucleoli (Wright-Giemsa stain, × 1,000).
6% of WBCs were blasts, and a bone marrow aspirate smear showed that blasts comprised up to 71% of the total nucleated cells (Fig. 1B). Immunophenotyping of bone marrow cells showed positive expression of myeloperoxidase (66%), CD13 (60%), CD33 (98%), CD117 (97%), and CD65 (39%). A cytogenetic analysis showed that the karyotype of the bone marrow cells was 46,XY,t(10;11)(p13;q21)[16]/46,XY[4]. A FISH analysis showed that rearrangements were absent in the following genes: promyelocytic leukemia–retinoic acid receptor alpha (PML–RARA), breakpoint cluster region–c-abl oncogene 1 receptor tyrosine kinase (BCR–ABL1), core–binding factor beta subunit (CBFB), MLL and runt–related transcription factor 1–runt–related transcription factor 1: translocated to 1 (RUNXI–RUNXIT1) (AML–ETO). Further, negative results were obtained for an analysis of fms–related tyrosine kinase 3 (FLT3) mutation. The patient was diagnosed with AML without maturation. He received induction and consolidation chemotherapy followed by allogenic peripheral blood stem cell transplantation. A 1-month follow-up bone marrow examination revealed successful engraftment without any evidence of relapse.

3. Reverse transcriptase-PCR and direct sequencing

Total RNA was extracted from fresh and cryopreserved bone marrow samples. CALM–AF10 fusion transcript was detected by reverse transcriptase–PCR (RT–PCR) using the forward primer CALM S1770 (5′–GCAATCTTGCGAT–CGGAAAT–3′) and the reverse primer AF10 AS559 (5′–CGATCTGCGGAACAGACTG–3′). The RT–PCR reaction mixture contained 2 μL of 10× reaction buffer, 2.5 mM MgCl2, 0.2 mM dNTPs, 25% dimethyl sulfoxide, 10 pmol of each primer, and 1 U of Taq DNA polymerase (Bioneer, Daejeon, Korea) in a total volume of 20 μL. The following cycling conditions were used: initial denaturation at 94°C for 8 min, 35 cycles of 94°C for 3 min, 60°C for 1 min and 72°C for 1 min, followed by a final elongation at 72°C for 10 min. PCR products were separated on 2% agarose gels and visualized by ethidium bromide staining. The PCR products were sequenced directly in both forward and reverse directions using a BigDye Terminator Cycle Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. Sequencing products were run on an ABI3700XL Genetic Analyzer (Applied Biosystems). The U937 cell line was used as a positive control, PCR primers for the HOXA and hDOT1L genes (Table 1) were designed on the basis of the known cDNA sequences.

The CALM–AF10 fusion transcript was detected by RT–PCR in samples obtained from both the patients (Fig. 2). In each case, sequencing analyses revealed breakpoints at nucleotides 2091 and 424 in CALM and AF10, respectively, which corresponded to the breakpoints in the U937 cell line. Both patient samples showed expression of the HOXAgene cluster and hDOT1L. In the first patient (case 1), the RT–PCR expression pattern of HOXA and hDOT1L genes was associated with the clinical outcome (Fig. 3).

**DISCUSSION**

The CALM–AF10 rearrangement was first observed in a patient with diffuse histiocytic lymphoma: the U937 cell line was established from this patient and was found to display many monocytic characteristics [11]. The CALM–AF10 rearrangement has been observed as a rare but recurring phenomenon in acute leukemias of several lineages, including myeloid, T-cell lymphoblastic, and megakaryocytic leukemias, as well as malignant lymphoma, and is considered to be indicative of a poor clinical outcome [2–4]. The incidence of CALM–AF10 fusion transcripts in acute leukemia has not been well documented, although one study showed that CALM–AF10 transcripts were present in 9% of 131 T-cell ALL patients and was the most common fusion
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Recently, CALM-AF10-positive leukemias were found to be characterized by the overexpression of HOXA5, HOXA9, HOXA10, and the BMI1 polycomb ring finger oncogene (BMI1) [8]. Okada et al. [9] reported that leukemic transformation by CALM–AF10 was mediated by the recruitment of the histone methyltransferase hDOT1L, which methylates H3K79. hDOT1L was also found to interact with an octapeptide motif and a leucine–zipper (OM–LZ) region of AF10; this interaction inhibits the nuclear export of CALM–AF10. Both hDOT1L–mediated methylation of H3K79, which results in the overexpression of several HOXA genes, and nuclear sequestration of CALM–AF10 contribute to leukemic transformation by CALM–AF10 [9, 12]. Among the upregulated HOXA genes, HOXA5 has been shown in a HOXA5–knockout mouse model to be critical for leukemic transformation by the CALM–AF10 fusion gene [9]. In the present report, the genes HOXA5, HOXA7, HOXA9, HOXA10, and hDOT1L were expressed in both the patients. Achievement of hematologic remission in case 1 was associated with the suppression of HOXA and hDOT1L gene expression; these genes were re–expressed upon relapse. HOXA gene expression is associated with early hematopoiesis and is generally downregulated during differentiation [13]. In CALM–AF10–positive leukemias, HOXA expression possibly contributes to leukemogenesis by inhibiting cellular differentiation. This behavior is characteristic of CALM–
API10–positive leukemias that show a mixed-lineage immunophenotype.

Increased expression of HOXA genes has also been described in acute leukemia with MLL-rearrangement and a SET nuclear oncogene–nucleoporin 214 kDa (SET–NUP214) fusion gene [14]. Moreover, like CALM–API10–positive leukemia, leukemic cells containing MLL–API10 fusion transcripts showed not only HOXA gene overexpression but also hDOT1L–mediated H3K79 methylation of HOXA genes. This finding indicates that CALM–API10– and MLL–API10–positive leukemias share a leukemic transformation mechanism involving hDOT1L and HOX gene activation [11]. Therefore, inhibition of hDOT1L could be a promising new therapeutic strategy for treating CALM–API10– and MLL–API10–positive leukemias.

In summary, we report 2 cases of CALM–API10–positive acute leukemia. The first patient (case 1) exhibited clinical characteristics of CALM–API10 typical of those previously reported, including young age, mixed–lineage immunophenotype, extramedullary involvement, and poor clinical outcome. The 2 patients had T-cell ALL and AML, and both showed the HOXA gene cluster and hDOT1L gene expression. Given the poor prognosis associated with conventional chemotherapy, CALM–API10–positive leukemias should be monitored by RT–PCR, even under conditions of clinical remission. The expression of HOXA genes could be useful as a molecular marker for residual disease after therapy.

REFERENCES


