Korean J Lab Med 2010;30:117-21
DOI 10.3343/kjlm.2010.30.2.117

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

Ji Young Huh, M.D.[1], Soie Chung, M.D.[2], Doyeun Oh, M.D.[3], Myung Seo Kang, M.D.[4], Hyeon-Seok Eom, M.D.[5,6], Eun Hae Cho, M.D.[7], Mi Hwa Han, M.S.[5], and Sun-Young Kong, M.D.[5,8]

Department of Laboratory Medicine[1], CHA Bundang Medical Center, CHA University, Seongnam; Department of Laboratory Medicine[2], Seoul National University Hospital, Seoul; Departments of Internal Medicine[3] and Laboratory Medicine[4], CHA Gangnam Medical Center, CHA University, Seoul; Hematologic Malignancies Branch[5], Research Institute and Hematology Oncology Clinic[6], National Cancer Center, Goyang; Greencross Reference Laboratory[7], Yongin; Department of Laboratory Medicine[8], National Cancer Center, Goyang, Korea

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*

INTRODUCTION

The translocation t(10;11)(p13;q14q21) is recurrent in ALL and AML [1]. It has also been described in acute megakaryoblastic, monocytic, and eosinophilic leukemias as well as malignant lymphomas [2-4]. This translocation fuses the clathrin assembly lymphoid myeloid leukemia (*CALM*) gene with the *AF10* gene, which are present on chromosomes 11 and 10, respectively. The *CALM-AF10* fusion gene comprises almost the complete open reading frames of *CALM* and *AF10* and encodes the CALM-AF10 fusion protein. Several different *CALM-AF10* fusion transcripts have been described, none of which have been clearly correlated with a specific clinical characteristic [1, 4]. Three and 4 different breakpoints in *CALM* and *AF10*, respectively, have been identified in *CALM-AF10* fusion transcripts [1, 2, 5]. *CALM-AF10*-positive hematologic malignancies

Received : June 25, 2009 Manuscript No : KJLM09-083
Revision received : March 9, 2010
Accepted : March 9, 2010
Corresponding author : Sun-Young Kong, M.D., Ph.D.
Hematologic Malignancies Branch, Division of Translational & Clinical Research II, Research Institute and Department of Laboratory Medicine, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang 410-769, Korea
Tel : +82-31-920-1735 Fax : +82-31-920-1738
E-mail : ksy@ncc.re.kr

*This work was supported by the National Cancer Center of Korea (Grant No.0810440).

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 *HOXA* 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 blood cell (WBC) and platelet counts of 10.4×10^9/L and 193×10^9/L, 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 0.9×10^9/L, and platelet count of 85×10^9/L. A peripheral blood smear revealed that

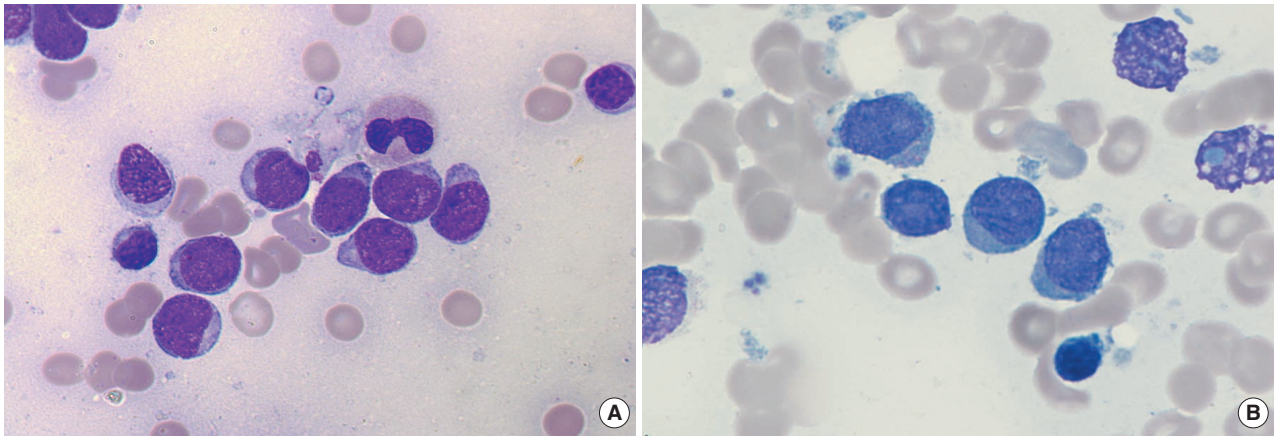

Fig. 1. Bone marrow aspiration shows several lymphoblasts in the first patient (case 1) (A) and myeloblasts in the second patient (case 2) (B) characterized by medium-sized nuclei and prominent nucleoli (Wright-Giemsa stain, ×1,000).

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-RUNX1T1*) (*AML1-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′-GCAATCTTGGCAT-CGGAAAT-3′) and the reverse primer AF10 AS559 (5′-CGATCATGCGGAACAGACTG-3′). The RT-PCR reaction mixture contained 2 μL of 10× reaction buffer, 2.5 mM $MgCl_2$, 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℃ for 8 min, 35 cycles of 94℃ for 3 min, 60℃ for 1 min and 72℃ for 1 min, followed by a final elongation at 72℃ 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.

Table 1. Primers used in PCR amplification

Name	Sequence
HOXA5_F	5′-GTCCACGCACTCTCCTCAG-3′
HOXA5_R	5′-CTTCATTCTCCGGTTTTGGA-3′
HOXA7_F	5′-TCAACAGCCCCCTTTATCAG-3′
HOXA7_R	5′-GCTCTTTCTTCCACTTCATGC-3′
HOXA9_F	5′-TAAACCTGAACCGCTGTCG-3′
HOXA9_R	5′-CATTTTCATCCTGCGGTTCT-3′
HOXA10_F	5′-GCTACTTCCGCCTTTCTCAG-3′
HOXA10_R	5′-GACGCTGCGGCTAATCTCTA-3′
hDOT1L_F	5′-CAAGTTCTCGCTGCCTCACT-3′
hDOT1L_R	5′-GTCCTGAGGGCTCAGCTTC-3′

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 *HOXA* gene 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

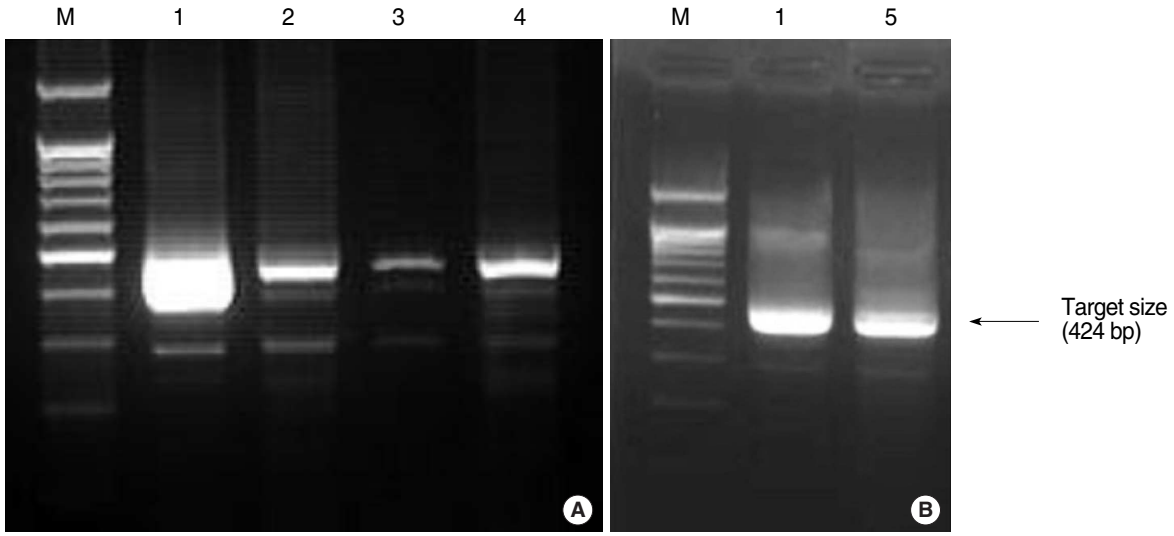

Fig. 2. Detection of the *CALM-AF10* fusion transcript by RT-PCR in bone marrow cells from cases 1 and 2 and the U937 cell line. A *CALM-AF10* fusion transcript of 424 bp was identified in samples of the first (case 1) (A) and second (case 2) (B) patients. Lane M: size marker; lane 1: U937 cell line; lanes 2-4: first patient (case 1) at diagnosis, hematologic remission, and relapse, respectively; lane 5; second patient (case 2) at diagnosis.
Abbreviation: CALM-AF10, clathrin assembly lymphoid myeloid leukemia-AF10.

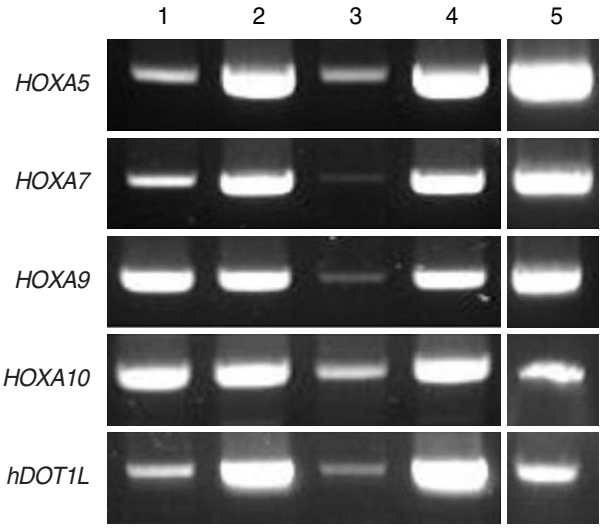

Fig. 3. Detection of the *HOXA* cluster gene and *hDOT1L* gene expression by RT-PCR in bone marrow cells from cases 1 and 2 and the U937 cell line. Lane 1: U937 cell line; lanes 2-4: first patient (case 1) at diagnosis, hematologic remission and relapse, respectively; lane 5: second patient (case 2) at diagnosis.
Abbreviations: HOXA, homeobox A; hDOT1L, human DOT1-like, histone H3 methyltransferase.

gene in patients with TCR$\gamma\delta$-lineage T-cell ALL [7].

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-*

AF10-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-AF10*-positive leukemia, leukemic cells containing *MLL-AF10* fusion transcripts showed not only *HOXA* gene overexpression but also hDOT1L-mediated H3K79 methylation of *HOXA* genes. This finding indicates that *CALM-AF10*- and *MLL-AF10*-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-AF10*- and *MLL-AF10*-positive leukemias.

In summary, we report 2 cases of *CALM-AF10*-positive acute leukemia. The first patient (case 1) exhibited clinical characteristics of *CALM-AF10* 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-AF10*-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

1. Bohlander SK, Muschinsky V, Schrader K, Siebert R, Schlegelberger B, Harder L, et al. Molecular analysis of the CALM/AF10 fusion: identical rearrangements in acute myeloid leukemia, acute lymphoblastic leukemia and malignant lymphoma patients. Leukemia 2000; 14:93-9.
2. Jones LK, Chaplin T, Shankar A, Neat M, Patel N, Samuel DP, et al. Identification and molecular characterisation of a CALM-AF10 fusion in acute megakaryoblastic leukaemia. Leukemia 2001;15:910-4.
3. Nakamura F, Maki K, Arai Y, Nakamura Y, Mitani K. Monocytic leukemia with CALM/AF10 rearrangement showing mediastinal emphysema. Am J Hematol 2003;72:138-42.
4. Caudell D and Aplan PD. The role of CALM-AF10 gene fusion in acute leukemia. Leukemia 2008;22:678-85.
5. Carlson KM, Vignon C, Bohlander S, Martinez-Climent JA, Le Beau MM, Rowley JD. Identification and molecular characterization of CALM/AF10fusion products in T cell acute lymphoblastic leukemia and acute myeloid leukemia. Leukemia 2000;14:100-4.
6. Kumon K, Kobayashi H, Maseki N, Sakashita A, Sakurai M, Tanizawa A, et al. Mixed-lineage leukemia with t(10;11)(p13;q21): an analysis of AF10-CALM and CALM-AF10 fusion mRNAs and clinical features. Genes Chromosomes Cancer 1999;25:33-9.
7. Asnafi V, Radford-Weiss I, Dastugue N, Bayle C, Leboeuf D, Charrin C, et al. CALM-AF10 is a common fusion transcript in T-ALL and is specific to the TCRgammadelta lineage. Blood 2003;102:1000-6.
8. Dik WA, Brahim W, Braun C, Asnafi V, Dastugue N, Bernard OA, et al. CALM-AF10+ T-ALL expression profiles are characterized by overexpression of HOXA and BMI1 oncogenes. Leukemia 2005;19: 1948-57.
9. Okada Y, Jiang Q, Lemieux M, Jeannotte L, Su L, Zhang Y. Leukaemic transformation by CALM-AF10 involves upregulation of Hoxa5 by hDOT1L. Nat Cell Biol 2006;8:1017-24.
10. Kim KH and Han JY. Simultaneous reverse transcription-polymerase chain reaction for detection of 7 gene rearrangements in acute leukemia. Korean J Clin Pathol 2001;21:24-33.
11. Sundstrom C and Nilsson K. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int J Cancer 1976; 17:565-77.
12. Sitwala KV, Dandekar MN, Hess JL. HOX proteins and leukemia. Int J Clin Exp Pathol 2008;1:461-74.
13. Okada Y, Feng Q, Lin Y, Jiang Q, Li Y, Coffield VM, et al. hDOT1L links histone methylation to leukemogenesis. Cell 2005;121:167-78.
14. Van Vlierberghe P, van Grotel M, Tchinda J, Lee C, Beverloo HB, van der Spek PJ, et al. The recurrent SET-NUP214 fusion as a new HOXA activation mechanism in pediatric T-cell acute lymphoblastic leukemia. Blood 2008;111:4668-80.