Molecular characterization and clinical course of *MLL-ACTN4* rearrangement in therapy-related hematologic malignancies

Rearrangement of the *MLL* gene located at 11q23 is found with over 70 different partner genes in approximately 5-10% of acute leukemia cases. While the most common is the *MLL-AF4* fusion resulting from the translocation t(4;11)(q21;q23), several rare and novel partner genes have been detected and characterized throughout the last decade.[1] A novel *MLL-ACTN4* was first reported by our group in a case of CD10- B-cell precursor (BCP) acute lymphoblastic leukemia (ALL).[2] The *ACTN4* gene located at chromosome 19q13.2 encodes an ubiquitously expressed actin-bundling protein associated with cell motility and cancer invasion.[3] Its mutation or deficiency is mainly associated with focal segmental glomerulosclerosis (FSGS);[4] however, its association with leukemogenesis is not clear.

To focus on the identification of *MLL-ACTN4* as a rare but recurrent MLL rearrangement, we present 2 cases of *MLL-ACTN4* rearrangement, and compare these patients with regard to diagnostic findings and clinical courses. To our knowledge, these are the only reported cases of *MLL-ACTN4* rearrangement so far, and thus a comprehensive analysis of these 2 *MLL-ACTN4* cases possibly provides an insight into this rare MLL rearrangement.

All analyses were carried out using bone marrow (BM) specimens obtained at the time of diagnosis. Both patients were analyzed by BM cytology and immunophenotyping. Fluorescence *in situ* hybridization (FISH) analysis using *MLL* probe (LSI dual color, break-apart probes) and a conventional karyogram were available in the second case. Long-distance inverse-polymerase chain reaction (LDI-PCR) at the Diagnostic Center of Acute Leukemia (DCAL, Frankfurt, Germany) was conducted in both cases. Microarray analysis was performed using Cytoscan 750K array (Affymetrix, Santa Clara, CA, USA) for the second case.

The first *MLL-ACTN4* case was a 69-year old woman who developed a secondary BCP-ALL after radio-chemotherapy treatment of a marginal zone lymphoma. Four cycles of chemotherapy under the FCM regimen (fludarabine, cyclophosphamide and mitoxantrone) and local radiotherapy of 30 Gy were used as treatment. This case was presented as a part of previous study.[2] Both LDI-PCR and reverse transcriptase-polymerase chain reaction (RT-PCR) confirmed reciprocal fusions of *MLL* and *ACTN4*, resulting from fusion of *MLL* intron 9 and *ACTN4* intron 1. The patient died one month after induction chemotherapy due to myocardial infarction. The second *MLL-ACTN4* case was found in a 5-year old male diagnosed with therapy-related myelodysplastic syndrome (t-MDS) after chemotherapy for previously diagnosed rhabdomyosarcoma. Treatment for rhabdomyosarcoma was chemotherapy using cisplastin, vincristin and etoposide. Karyotype was 46,XY,der(6)t(6;15)(p22;q21),t(11;19)(q23;q13.1) in all 20 cells analyzed (Figure 1). MLL FISH result was nuc ish (nuclear *in situ* hybridization) (MLLx2)(5'MLL sep 3'MLLx1) in 191 out of 200 cells observed (95.5%) (Figure 1). The LDI-PCR identified genomic fusions of both the *MLL-ACTN4* and its reciprocal *ACTN4-MLL* allele, resulting from fusion of *MLL* intron 11 and *ACTN4* intron 1 (Figure 1). No RT-PCR analysis was possible. Microarray result was arr 6p25.2p22.2(381, 135-28, 638, 046)x1, 15q21.3q26.3(59, 048, 632-102, 429, 040)x3, showing about 28Mb loss at 6p25.2p22.2 and about 43Mb gain at

Table 1. Laboratory and clinical features of the 2 patients with *MLL-ACTN4* rearrangement.

	Case 1	Case 2
Gencer/Age (years)	F/69	M/5
Hematologic diagnosis	B-precursor ALL (secondary ALL)	t-MDS
Previous diagnosis	Marginal zone lymphoma (diagnosed 14 months before ALL diagnosis, treated with 4xFCM and radiation)	Rhabdomyosarcoma (diagnosed 36 months before diagnosis of t-MDS (RAEB-II), treated with cisplastin, vincristin and etoposide)
CBC at diagnosis	WBC 2,400/uL; Hb 8.5 g/dL; PLT 27x10^9/L	WBC 4,360 /uL; Hb 8.6 g/dL; PLT 95x10^9/L
LDH at diagnosis	1699 U/l	594 U/l
Immunophenotype results	CD19$^+$, CD10$^-$, CD22$^+$, cyCD22$^+$, CD33$^+$, CD34$^+$, HLA-DR$^+$, TdT$^+$, CD117$^-$, sIg$^-$, CD38$^-$, CD138$^-$	MPO$^+$, CD13$^+$, CD33$^+$, CD15$^+$, CD64$^+$, CD65w$^+$, HLA-DR$^+$ CD3$^-$, CD79a$^-$, CD34$^-$, CD14$^-$, CD117$^-$, CD41$^-$, CD2$^-$, CD5$^-$, CD7$^-$, CD10$^-$, CD19$^-$, CD20$^-$
Chromosome study	NA	46,XY,der(6)t(6;15)(p22;q21),t(11;19)(q23;q13.1)[20]
MLL FISH analysis	NA	nuc ish(MLLx2)(5'MLL sep 3'MLLx1)[191/200]
RT-PCR	Both reciprocal fusions detected	NA
LDI-PCR	MLL-ACTN4, ACTN4-MLL (MLL: Intron 9, ACTN4: Intron 1)	MLL-ACTN4, ACTN4-MLL (MLL: Intron 11, ACTN4: Intron 1)
Microarray study	NA	arr 6p25.2p22.2(381,135-28,638,046)x1, 15q21.3q26.3(59,048,632-102,429,040)x3
Treatment	First induction according to GMALL Elderly protocol	BH-AC, double UCBT
Prognosis (survival year)	Died of myocardial infarction one month after therapy	Alive after diagnosis (3 years)

M: male; F: female; t-MDS: therapy-related myelodysplastic syndrome; B-ALL: B-cell acute lymphoblastic leukemia; FCM: fludarabine+cyclophosphamide +mitoxantrone; CBC: complete blood count; WBC: white blood cell; Hb: hemoglobin; PLT: platelet; FISH: fluorescence in situ hybridization; RT-PCR: reverse transcriptase-polymerase chain reaction; NA: not available; LDI-PCR: long-distance inverse-polymerase chain reaction; BH-AC: N4-behenoyl-1-beta-D-arabinofuranosyl-cytosine; UCBT: umbilical cord blood transplantation, GMALL: German Multicenter ALL Study Group.

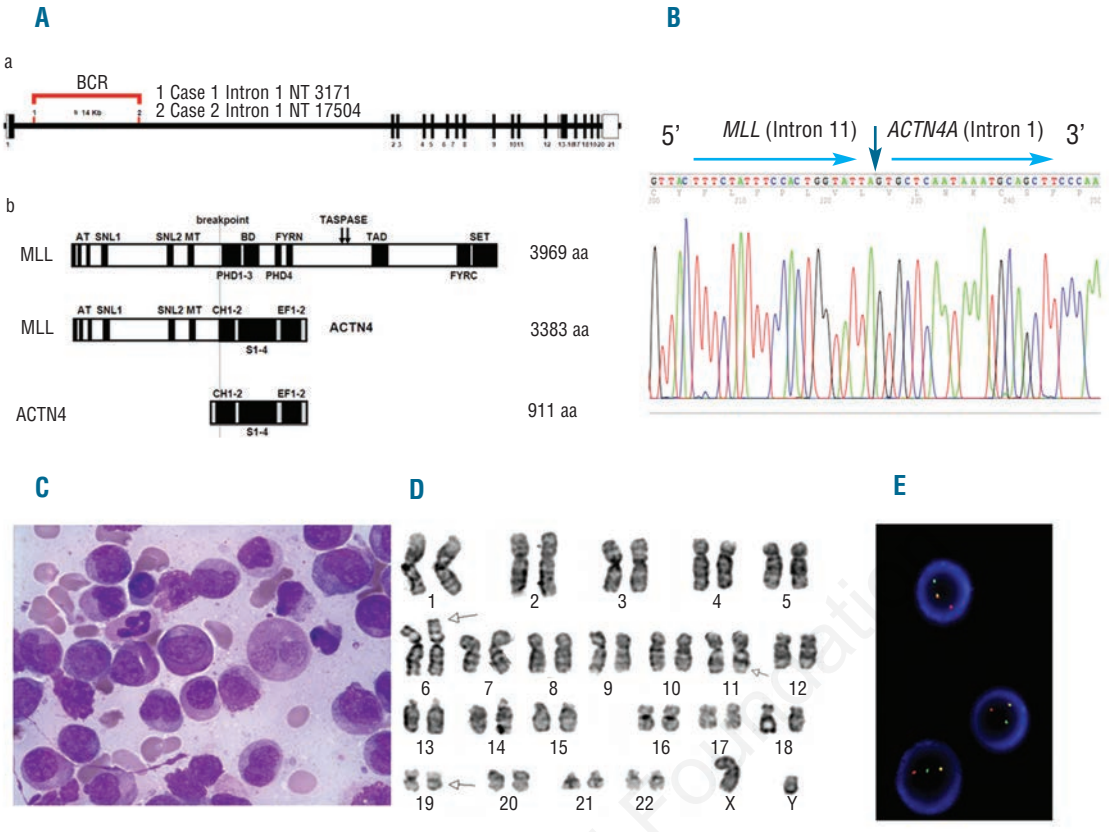

Figure 1. A. (a) Breakpoint cluster regions (BCRs) of the ACTN4 gene. (b) Size and location of functional domains of the MLL wt, ACTN4 wt, and of the MLL-ACTN4 fusion protein. (B~E; case 2). **B.** Identification of genomic fusion breakpoint between intron 11 of MLL and intron 1 of ACTN4. **C.** Bone marrow mophology showing myelodysplastic features. **D.** Giemsa banded karyogram, abnormal chromosomes indicated by arrows. **E.** Flouresecence *in situ* hybridization (FISH) image showing break-apart MLL signals. AT: AT hook; SNL: subnuclear localization; MT: methyltransferase; BD: binding domain; TAD: transcriptional activation domain; PHD: plant homeo domain; SET: Su(var)3e9, Enhancer-of-zeste, Trithorax; CH: calponin-homology domain; S: spectrin-like repeats; EF: EF-hand motif.

15q21.3q26.3. The patient is alive three years after diagnosis, and remains in remission. A detailed description of the laboratory and clinical features of these 2 cases is shown in Table 1.

Based on our findings, *MLL-ACTN4* rearrangements appear to be therapy-related. Use of topoisomerase-II inhibitors, such as mitoxantrone and etoposide, is likely to be responsible for therapy-related hematologic malignancies with MLL rearrangements, especially involving chromosome bands 11q23 or 21q22.[5] Clinical and laboratory data from both cases suggest the likelihood of cytotoxic (radio-)chemotherapy being responsible for the occurrence of these leukemias. The *ACTN4* gene is composed of 21 exons and has a coding capacity of 911 amino acids (Figure 1). In both patients, interestingly, the breakpoints were localized in *ACTN4* intron 1 which leads to an in-frame fusion of nearly the entire *ACTN4* open reading frame (except the first 54 amino acids encoded by exon 1) to the N-terminal portion of *MLL* gene (Figure 1). Since the *ACTN4* as well as *ENL* and *ELL* genes are located on chromosome 19, albeit on different arms, confirmatory tests should be conducted to unambigously distinguish between either of these three possibilities in patients with a t(11;19) translocation. In circumstances of t(11;19) with a negative result for *MLL-ENL* or *MLL-ELL*, a genomic breakpoint analysis using LDI-PCR should be able to readily identify *MLL-ACTN4* or other rare *MLL* rearrangements (*MLL-ACER1*, *MLL-MYO1F*).[1,6] As *MLL-ACTN4* rearrangements are rarely found, little is known of its response to therapy and prognosis. Additional reports in the future will shed light on the understanding of leukemogenesis of this rare *MLL*-related fusion gene.

John Jeongseok Yang,[1,2] Tae Sung Park,[2] Seung-Tae Lee,[3]* Ja-Young Seo,[3] Seung Hwan Oh,[4] Eun Hae Cho,[5] Thomas Burmeister,[6] Wolf-Dieter Ludwig,[7] Claus Meyer,[8] Rolf Marschalek,[8] Hee-Jin Kim,[3] and Sun-Hee Kim[3]*

**TSP, STL, and SHK equally contributed to this work.*

[1]Department of Medicine, Graduate School, Kyung Hee University, Seoul, Korea; [2]Department of Laboratory Medicine, School of Medicine, Kyung Hee University, Seoul, South Korea; [3]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea; [4]Department of Laboratory Medicine, Inje University College of Medicine, Busan, South Korea; [5]Green Cross Genome, Yongin, South Korea; [6]Klinik für Hämatologie, Charité, 12200 Berlin, Germany; [7]Department of Hematology, Oncology, and Tumor Immunology at the HELIOS Klinikum Berlin-Buch, Schwanebecker Chaussee 50, 13125 Berlin, Germany; and [8]Institute of Pharmaceutical Biology, ZAFES, Diagnostic Center of Acute Leukemia, Goethe-University of Frankfurt, Germany

Correspondence: 153jesus@hanmail.net
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