Genomic breakpoints and clinical features of *MLL-TET1* rearrangement in acute leukemias

Recent rapid developments in new technology such as whole genome/exome sequencing have revealed that novel mutations in genes such as *DNMT3A*, *IDH1*, *IDH2* and *TET2* contribute to the main process of leukemogenesis in patients with normal karyotype acute myeloid leukemia (AML).[1] Among these genes, *TET2*, a *TET* family gene located on 4q24, is important because of its high frequency of mutations in myeloid neoplasms such as myeloproliferative neoplasm (MPN), myelodysplastic syndromes (MDS), MDS/MPN, and AML.[2] In contrast, the *TET1* gene, first discovered in 2002 and located on 10q22, is rarely reported as a translocation partner for *MLL* rearrangements in acute leukemias.[3,4] Only 10 cases have been reported with chromosomal translocations t(10;11)(q22;q23) interchangeable with *MLL-TET1* fusions.[4-11] There has been little research into the common features of *MLL-TET1* rearrangements. Here we provide information about molecular and clinical characterization of *MLL-TET1* rearrangements diagnosed in 3 AML patients.

Using the long-distance inverse-polymerase chain reaction (LDI-PCR) method, 3 AML patients with *MLL-TET1* rearrangements were identified between January 2005 and December 2011 at the Diagnostic Center of Acute Leukemia (DCAL) in Frankfurt. All 3 cases were sent to the DCAL because of positive results for *MLL* rearrangements by conventional cytogenetics and fluorescence *in situ* hybridization (FISH), but routine analysis did not reveal the fusion partner of *MLL*. The 3 patients were from different institutions in Korea, Germany and France. Case 2 of this study was briefly mentioned (for rearrangement type) in a previous study.[12]

The results of clinical parameters, cytogenetic studies, FISH, reverse transcriptase-PCR and LDI-PCR analyses on bone marrow specimens at the time of diagnosis for 3 new AML cases are shown in Table 1. Including 3 new cases, this research analyzes a total of the 13 acute leukemia cases of t(10;11)(q22;q23) known so far, detected in 11 AML and 2 ALL patients. We summarized the published data to evaluate patients' characteristics (Table 2).[4-11] A molecular characterization at the genomic or transcript level was performed only for a subset of patients. Eight of 11 AML patients (72.7%) were classified as FAB-M4/M5 subtypes; 7 men and 5 women with a median age of 38.0 years (range 1 month to 67 years). For one patient, no clinical data could be retrieved.[11] Of 9 patients with available chromosome study results, 3 had a sole chromosomal abnormality of t(10;11), and the remaining 6 had accompanying additional chromosomal abnormalities. *MLL-TET1* rearrangement was confirmed in 8 of 13 cases and chromosomal breakpoints were also identified in these cases (Tables 1 and 2). All genomic breakpoints within the *TET1* gene were identified in an approximately 17 kb genomic region flanked by *TET1* exons 8 and 12 (Figure 1). But most characterized breakpoints (5 of 7 cases) were mapped to intron 8. Because only 7 of the 13 patients had available clinical outcomes, a sufficient amount of data is not available. Nevertheless, the results showed that 6 patients had complete remission. Five patients, including one who did not reach complete remission, died at an average of 14.5 months after initial diagnosis, showing little difference with the known poor prognosis of patients with *MLL* rearrangements.

TET1 encodes a protein of 2136 amino acids with a molecular mass of 235.3 kDa. TET1 contains a CXXC domain at position 583–624, a coiled-coil region near the C-terminus (position 2,062–2,091), and three candidate bipartite nuclear localization signals.[4,7] Lorsbach *et al.*[7] found that the expression of TET1 within the hematolymphoid system was limited to spleen, with no detectable expression in bone marrow or peripheral blood leukocytes. MLL-TET1 fusion proteins are predicted to have a molecular mass of 204.4 kDa and to retain the AT hooks, subnuclear localization domains, and CXXC motif of MLL, as well as the coiled-coil region and the most C-terminal

No.	Case	Intron	Nucleotide
1	Ono *et al.* (2002)[4]	8	1299
2	Lorsbach *et al.* (2003)[7]	8	2249
3	Case 2	8	5321
4	Case 1	8	6579
5	Burmeister *et al.* (2009)[5]	8	8107
6	Case 3	10	1373
7	Burmeister *et al.* (2009)[5]	11	4050

Figure 1. Schematic representation of *TET1* intron breakpoints on 4 reported cases and 3 new cases using genomic analyses.

Table 1. Characterization of 3 new acute myeloid leukemia cases with *MLL-TET1* gene rearrangement.

	Case 1	Case 2	Case 3
Gender/Age	M/39	F/60	M/18
Country	Korea	Germany	France
Diagnosis	AML (FAB M4)	AML (FAB M4)	AML (therapy-related : VP16 for Ewing sarcoma)
Peripheral blood finding at diagnosis	Hb: 9.6 g/dL PLT: 52×10^9/L WBC: 9.1×10^9/L	Hb: 9.5 g/dL PLT: 55×10^9/L WBC: 89.5×10^9/L	Hb: 5.4 g/dL PLT: 12×10^9/L WBC: 12.9×10^9/L
Blast counts in PB and BM	73% and 40%	BM: 90%	1% and 21%
BM interpretation with cellularity	85% cellular marrow. Erythroid dysplasia (binucleation, nuclear budding, intercytoplasmic bridge, megaloblastic changes).	Bone marrow hypercellularity with myelo-monocytic blast population.	Erythroid and granulocytic dysplasia
Immunophenotype	MPO^+, $CD34^+$, $CD117^+$, TdT^-	NA	MPO^+, butyrates $^+$, $CD34^+$, $CD117^-$, $CD33^+$, $CD13^+$
Chromosome study	46,XY,t(10;11)(q22;q23)[4]/47,idem,+21[16]	46,XX,t(10;11)(q22;q23)[21]	46,XY,t(10;11)(q22;q23)[2]/46,idem, del(6)(p21)[18]
MLL FISH results	nuc ish (*MLL*x2)(5'*MLL* sep 3'*MLL*x1) [495/500]	nuc ish (*MLL*x2) (5'*MLL* sep 3'*MLL*x1) [80/100]	nuc ish (*MLL*x2)(5'MLL sep 3'*MLL*x1)[95/100]
Method for *MLL/TET1* rearrangement	LDI-PCR, RT-PCR	LDI PCR	LDI PCR
Breakpoints (*MLL/TET1*)	*MLL* intron 8/ *TET1* intron 8	*MLL* intron 9/ *TET1* intron 8	*MLL* intron 10/ *TET1* intron 10
Clinical outcome	NA	Reached CR. Bone marrow transplanted. Relapsed 18 months after diagnosis and died 21 months after diagnosis.	Surviving 20 months after diagnosis.

M: male; F: female; FAB: French-American-British classification; PB: peripheral blood; BM: bone marrow; AML: acute myeloid leukemia; NA: not available; FISH: fluorescence in situ *hybridization; RT-PCR: reverse transcription-polymerase chain reaction; LDI-PCR: long-distance inverse PCR; CR: complete remission. Hb: hemoglobin; PLT: platelets; WBC: white blood cell count.*

Table 2. Summary of previously reported t(10;11) (q22;q23) cases.

Patient N.	Gender/Age	Diagnosis (FAB)	Chromosomal study	Method for *MLL/TET1* rearrangement	Genomic DNA breakpoint	cDNA breakpoint	Clinical outcome	Bone marrow dysplasia	References
1	NA	AML (M5)	t(10;11)(q22;q23)	NA	NA	NA	NA	NA	Thirman *et al.*[11]
2	M/46	AML (M5a)	46,XY,t(10;11)(q22;q23), add(13)(p11)/46,XY,t (10;11),der(6)t(6;?9) (p22;?q21)	NA	NA	NA	Achieved CR, EFS 6.7 mo, Survival 6.7 mo	NA	Harrison *et al.*[10]
3	F/30	AML (M5b)	46,XX,-10,der(11)t(10;11) (q22;q23),add(22))q13),+r	NA	NA	NA	Achieved CR, EFS 6.4 mo, Survival 10.6 mo	NA	Harrison *et al.*[10]
4	F/35	AML (M4)	51,XX,+8,t(10;11)(q22;q23), +13,+18,+18,+20[10]/46,XX[5]	FISH	NA	NA	Not achieved CR, survival 8 mo, died of infection	Mild dyshemopoiesis	Aventin *et al.*[9]
5	M/1mo	AML (M4)	46,XY,del(5)(q15),t(10;11) (q22;q23)[19] /46,XY[1],	NA	NA	NA	NA	NA	Kim *et al.*[8]
6	M/67	AML (M2)	46,XY, t(10;11)(q22;q23)	Southern blot, RT-PCR cDNA panhandle PCR	*MLL* intron 9/*TET1* intron 8	*MLL* exon 8/*TET1* exon 10 *MLL* exon 9/*TET1* exon 9	Achieved CR, EFS 24 mo, survival 26 mo, died of infection	Trilineage dysplasia	Ono *et al.*[4]
7	M/8	AML (M4)	46,XY, t(10;11)(q22;q23)	RT-PCR, LDI-PCR	*MLL* intron 6/*TET1* intron 8	*MLL* exon 9/*TET1* exon 9	CR for 24 months +	NA	Lorsbach *et al.*[7]
8	M/54	AML (M2)	NA	RT-PCR	NA	MLL exon 8/*TET1* (NA)	NA	NA	Shih *et al.*[6]
9	F/57	ALL	NA	RT-PCR, LDI-PCR	*MLL* intron 10/*TET1*	*MLL* exon 9/*TET1* exon 9 intron 8	NA	NA	Burmeister *et al.*[5]
10	F/67	ALL	NA	RT-PCR, LDI-PCR	*MLL* intron 7/*TET1* intron 11	*MLL* exon 7/*TET1* exon 12	NA	NA	Burmeister *et al.*[5]

N: number; M: male; F: female; mo: month; FAB: French-American-British classification; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; NA: not available; FISH: fluorescence in situ *hybridization; RT-PCR: reverse transcription-polymerase chain reaction; LDI-PCR: long-distance inverse PCR; EFS: event-free survival; CR: complete remission.*

nuclear localization domain of TET1.[7] The role of the *MLL-TET1* fusion gene in leukemogenesis is unknown, as is the physiological role of *TET1*.[5] Further clinical and molecular research on acute leukemias with *MLL-TET1* rearrangements is necessary, including functional study of the *MLL-TET1* fusion gene, to define the role of such rare *MLL*-related fusion genes in leukemogenesis.

There is a need to collect and report recurrent genomic or cytogenetic aberrations since such data on leukemia patients with particular genetic abnormalities improve our current understanding of genetic and clinical features in acute leukemias. Further research on the biological and prognostic effects of *MLL-TET1* rearrangements using animal models or human leukemia samples is also required in the near future.

Sang-Guk Lee,[1,2] Sun Young Cho,[3] Min Jin Kim,[3] Seung Hwan Oh,[4] Eun Hae Cho,[5] Sanggyu Lee,[6] Eun Jung Baek,[7]* Jung Hye Choi,[8] Stefan K. Bohlander,[9] Laurence Lode,[10] Steven Richebourg,[10] Hwi-Joong Yoon,[11] Rolf Marschalek,[12] Claus Meyer,[12] and Tae Sung Park[3]**

[1]Department of Laboratory Medicine, Armed Forces Capital Hospital, Seongnam, Korea; [2]Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea; [3]Department of Laboratory Medicine, School of Medicine, Kyung Hee University, Seoul, Korea; [4]Department of Laboratory Medicine, Inje University College of Medicine, Busan, Korea; [5]Greencross Reference Laboratory, Yongin, Korea; [6]School of Life Science and Biotechnology, Kyungpook National University, Daegu, Korea; [7]Department of Laboratory Medicine, Hanyang University Kuri Hospital, Kuri, Korea; [8]Department of Internal Medicine, Hanyang University Kuri Hospital, Kuri, Korea; [9]Laboratory of Leukemia Diagnostics, Department of Medicine III, University of Munich Hospital, Munich, Germany; [10]Service de Biologie/Hématologie, CHU, Nantes, France; [11]Department of Hematology-Oncology, School of Medicine, Kyung Hee University, Seoul, Korea; and [12]Institute of Pharmaceutical Biology, ZAFES, Diagnostic Center of Acute Leukemia, Goethe-University of Frankfurt, Germany

* SYC, EJB and TSP equally contributed to this work.

Correspondence: Tae Sung Park. 153jesus@hanmail.net
doi:10.3324/haematol.2012.076323

Funding: this research was supported by a grant from the Kyung Hee University in 2011 (KHU-20110932) to TSP, by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0026054) to TSP, and by German Pediatric Cancer Foundation (DKS-2011.09) to RM and CM. This work was also supported by a grant from the National Research Foundation of Korea (2010-0011200) to EJB.

Key-words: genomic breakpoints, MLL, TET1, acute leukemia

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Shen Y, Zhu YM, Fan X, Shi JY, Wang QR, Yan XJ, et al. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. Blood. 2011;118(20):5593-603.
2. Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, et al. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci USA. 2002;99(26):16899-903.
3. Abdel-Wahab O, Mullally A, Hedvat C, Garcia-Manero G, Patel J, Wadleigh M, et al. Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood. 2009;114(1):144-7.
4. Ono R, Taki T, Taketani T, Taniwaki M, Kobayashi H, Hayashi Y. LCX, leukemia-associated protein with a CXXC domain, is fused to MLL in acute myeloid leukemia with trilineage dysplasia having t(10;11)(q22;q23). Cancer Res. 2002;62(14):4075-80.
5. Burmeister T, Meyer C, Schwartz S, Hofmann J, Molkentin M, Kowarz E, et al. The MLL recombinome of adult CD10-negative B-cell precursor acute lymphoblastic leukemia: results from the GMALL study group. Blood. 2009;113(17):4011-5.
6. Shih LY, Liang DC, Fu JF, Wu JH, Wang PN, Lin TL, et al. Characterization of fusion partner genes in 114 patients with de novo acute myeloid leukemia and MLL rearrangement. Leukemia. 2006;20(2):218-23.
7. Lorsbach RB, Moore J, Mathew S, Raimondi SC, Mukatira ST, Downing JR. TET1, a member of a novel protein family, is fused to MLL in acute myeloid leukemia containing the t(10;11)(q22;q23). Leukemia. 2003;17(3):637-41.
8. Kim HJ, Cho HI, Kim EC, Ko EK, See CJ, Park SY, et al. A study on 289 consecutive Korean patients with acute leukaemias revealed fluorescence in situ hybridization detects the MLL translocation without cytogenetic evidence both initially and during follow-up. Br J Haematol. 2002;119(4):930-9.
9. Aventin A, La Starza R, Martinez C, Wlodarska I, Boogaerts M, Van den Berghe H, et al. Involvement of MLL gene in a t(10;11)(q22;q23) and a t(8;11)(q24;q23) identified by fluorescence in situ hybridization. Cancer Genet Cytogenet. 1999;108(1):48-52.
10. Harrison CJ, Cuneo A, Clark R, Johansson B, Lafage-Pochitaloff M, Mugneret F, et al. Ten novel 11q23 chromosomal partner sites. European 11q23 Workshop participants. Leukemia. 1998;12(5):811-22.
11. Thirman MJ, Gill HJ, Burnett RC, Mbangkollo D, McCabe NR, Kobayashi H, et al. Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. N Engl J Med. 1993;329(13):909-14.
12. Meyer C, Kowarz E, Hofmann J, Renneville A, Zuna J, Trka J, et al. New insights to the MLL recombinome of acute leukemias. Leukemia. 2009;23(8):1490-9.