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Original Article

TET2 Mutation in Adult T-Cell Leukemia/Lymphoma

Kazuya Shimoda, Kotaro Shide, Takuro Kameda, Tomonori Hidaka, Yoko Kubuki, Ayako Kamiunten, Masaaki Sekine, Keiichi Akizuki, Haruko Shimoda, Takumi Yamaji, Kenichi Nakamura, Hiroo Abe, Tadashi Miike, Hisayoshi Iwakiri, Yoshihiro Tahara, Mitsue Sueta, Shojiro Yamamoto, Satoru Hasuike, Kenji Nagata, and Akira Kitanaka

Loss-of-function of ten-eleven translocation-2 (TET2) is a common event in myeloid malignancies, and plays pleiotropic roles, including augmenting stem cell self-renewal and skewing hematopoietic cells to the myeloid lineage. *TET2* mutation has also been reported in lymphoid malignancies; 5.7～12% of diffuse large B-cell lymphomas and 18～83% of angioimmunoblastic T-cell lymphomas had *TET2* mutations. We investigated *TET2* mutations in 22 adult T-cell leukemia/lymphoma (ATLL) patients and identified a missense mutation in 3 cases (14%). *TET2* mutation occurred in a number of ATLL patients and was likely involved in their leukemogenesis. 〔*J Clin Exp Hematop 55(3) : 145-149, 2015*〕

Keywords: ATLL, TET2, polymorphism

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATLL) is an aggressive T-cell neoplasm caused by human T-cell leukemia virus type 1 (HTLV-1).[1-3] Retroviral HTLV-1 is transmitted to neonatal $CD4^+$ T-cells through breast-feeding by cell-to-cell transmission. Proviral DNA is generated from genomic viral RNA, followed by random integration into the host genome. After a latency period of 40-60 years, gene mutations accumulate in HTLV-1-infected $CD4^+$ T-cells, and ATLL develops in about 3-5% of HTLV-1-infected individuals.

Somatic mutations in *Ten-Eleven Translocation-2* (*TET2*) were first identified in myeloproliferative neoplasms (MPN) and myelodysplastic syndromes.[4,5] TET2 plays a key role in the conversion of 5-methyl-cytosine to 5-hydroxymethyl cytosine (5-hmC), and *TET2* mutations, including deletions, missense, nonsense, and frameshift mutations, were shown to result in loss-of-function of TET2 and a marked reduction in global levels of 5-hmC.[6] We and others reported that *TET2*-deficient hematopoietic stem cells showed increased self-renewal ability and exhibited a competitive growth advantage over wild-type hematopoietic stem cells.[7-10]

In addition to myeloid malignancies, *TET2* mutations have been detected in B-lineage and T-lineage lymphoid malignancies.[11-15] Of these, *TET2* was most frequently mutated in angioimmunoblastic T-cell lymphomas and "Th follicular (T_{FH})-like" peripheral T-cell lymphomas, not otherwise specified.[12-14] Because ATLL is a type of peripheral T-cell lymphoma, we investigated 22 ATLL patients for *TET2* mutation and identified 3 patients with missense mutations.

MATERIALS AND METHODS

Patients and tumor samples

A series of 22 ATLL patients with frozen tumor cell samples available were selected. The specimens were collected between 2005 and 2011, with 70% between 2008 and 2011. These samples were not included in the sample set used for the genome-wide analysis of ATLL we recently published.[16] Medical records were reviewed for clinical data. Diagnosis of ATLL was based on clinical features, cytologically-proven mature T-cell malignancy, the presence of anti-HTLV-1 antibodies, and monoclonal integration of HTLV-1 proviral DNA into tumor cells, as confirmed by Southern blotting. This study was approved by the Research Ethics Committee of University of Miyazaki.

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Department of Gastroenterology and Hematology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan
Corresponding Author: Prof. Kazuya Shimoda, Department of Gastroenterology and Hematology, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan
E-mail: kshimoda@med.miyazaki-u.ac.jp

TET2 genotyping

DNA was extracted from frozen cells using a standard protocol. The coding sequence of the *TET*2 gene (exons 3 through 10) was amplified by the polymerase chain reaction (PCR) method with the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). The sequences of the PCR primers used for *TET2* were designed as described in a previous report,[17] and purchased from Hokkaido System Science Co., Ltd. Nucleotide sequences were determined by fluorescent dye chemistry sequencing with an ABI PRISM3000 DNA Analyzer (Applied Biosystems and analyzed with Sequencing Analysis software (Applied Biosystems). The presence of mutations or single nucleotide polymorphisms was determined by referencing the assembled sequence in the Ensembl genome database.[18]

Interpretation of mutations

To predict whether the identified TET2 mutations may have deleterious effects, we used two online software programs, Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org/webcite)[19] and Polymorphism Phenotyping v2 (Polyphen-2; http://genetics.bwh.harvard.edu/pph2/webcite).[20] SIFT uses sequence homology to predict amino acid substitutions that may affect protein function. SIFT scores range from 0 to 1, and scores < 0.05 suggest that the amino acid change is not tolerated.[19] PolyPhen-2 uses straightforward physical and comparative considerations to predict the possible impact of an amino acid substitution on the structure and function of a human protein. Polyphen-2 scores > 0.85 are interpreted as probably damaging, and scores 0.15-0.85 as possibly damaging.[20]

RESULTS

Subject characteristics are listed in Table 1. Of the 22 patients, 15 were men and 7 were women. The median age was 67.5 years (range, 51-83). Based on the Shimoyama criteria,[21] 7 cases were diagnosed as lymphoma-type ATLL, and 14 cases with more than one of the following observations were diagnosed as acute-type ATLL: lactate dehydrogenase greater than twice the normal upper limit, Ca^{2+} > 11.0 mg/dL, and multiple organ ATLL involvement. One case was not classified due to the lack of lymphocyte-related data. ATLL cells were obtained from lymph nodes in 17 cases, from a skin tumor in 1 case, from sinusoid tumors in 2 cases, and from subcutaneous tumors in 2 cases. The surface markers of the ATLL cells were analyzed by flow cytometry in 13 cases, and by immunohistochemistry in 11 cases; all ATLL cells were positive for CD3. ATLL cells were $CD4^+$ $CD8^-$ in 12 cases, $CD4^+CD8^+$ in 5 cases, $CD4^-CD8^+$ in 1 case,

Table 1. Profiles and clinical data for each ATLL case

Case no.	Age (y)	Sex	WBC ($\times 10^9$/L) (5.0-9.0)	Lymphocyte ($\times 10^9$/L)	Abnormal lymphocyte (%)	LDH (IU/L) (119-229)	Ca^{2+} (mg/dL) (8.7-10.3)	sIL-2R (U/mL) (145-519)	Phenotype of leukemic cells in the sample (%)				Clinical type	Samples	The proportion of infiltrated ATLL cells in the sample by pathological examination (%)
									CD3	CD4	CD8	CD25			
1	65	F	19.3	9.0	32	892	22.2	70,900	95.4	96.4	93.8	92.2	Acute	LNs	n.d.
2	60	M	10.3	1.2	26	227	9.7	6,580	+	±	±	n.d.	Acute	LNs	90～100
3	72	M	4.0	0.2	4	725	9.0	69,300	63.9	65.3	7.1	86.8	Acute	LNs	n.d.
4	74	M	6.8	1.3	0	222	8.8	8,700	+	+	+	n.d.	Lymphoma	LNs	90～100
5	75	F	5.3	n.d.	n.d.	328	9.6	n.d.	+	+	−	n.d.	Unknown	LNs	90～100
6	51	M	23.9	3.6	53	1,094	8.3	30,800	+	+	−	n.d.	Acute	Skin tumor	10
7	68	M	6.3	2.2	0	374	10.2	3,320	+	n.d.	n.d.	n.d.	Acute	Sinusoid tumor	90～100
8	75	M	7.5	1.7	0	251	9.4	3,340	+	+	−	n.d.	Acute	Subcutaneous tumor	90～100
9	71	M	33.8	24.7	0	960	9.4	71,100	97.8	0.6	5.2	3.7	Acute	LNs	n.d.
10	59	F	3.9	0.3	2	884	9.3	11,900	59.2	26.9	29.2	n.d.	Acute	LNs	n.d.
11	58	M	6.3	1.8	0	268	9.0	21,700	90.5	97.0	70.2	79.3	Lymphoma	LNs	n.d.
12	56	F	2.6	0.7	0	178	9.5	641	+	+	n.d.	n.d.	Acute	Subcutaneous tumor	50
13	83	M	11.0	2.9	26	1,114	10.7	30,200	+	+	−	n.d.	Acute	LNs	90～100
14	72	F	9.0	1.8	0	239	9.2	21,500	97.5	96.3	0.5	n.d.	Lymphoma	LNs	n.d.
15	73	F	6.7	1.4	2	272	9.8	6,910	74.0	48.7	56.4	54.1	Acute	Sinusoid tumor	n.d.
16	76	M	5.2	1.6	0	1,802	9.8	59,200	99.1	88.2	0.0	n.d.	Acute	LNs	n.d.
17	65	M	7.3	1.3	0	299	9.6	4,770	72.2	87.6	0.3	n.d.	Lymphoma	LNs	n.d.
18	51	F	5.6	0.8	0	272	9.6	1,330	80.1	95.6	4.7	31.7	Lymphoma	LNs	n.d.
19	70	M	6.2	0.5	0.5	442	9.9	12,900	60.9	97.1	0.0	33.1	Lymphoma	LNs	n.d.
20	45	M	4.4	2.1	0	187	9.6	840	95.9	17.6	66.8	n.d.	Lymphoma	LNs	n.d.
21	67	M	15.2	3.0	49	672	9.2	24,800	69.7	61.1	7.9	35.4	Acute	LNs	n.d.
22	62	M	54.8	5.8	22	5,626	9.8	35,600	+	+	−	n.d.	Acute	LNs	90～100

The result of immunohistochemical staining of tumors samples are shown as +, ±, or −, corresponding to positive, weak positive, or negative, respectively. The Arabic number indicates the positive rate (%) of cell surface antigen by means of flow cytometric analysis. ATLL, adult T-cell leukemia/lymphoma; WBC, white blood cell; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin-2 receptor; LNs, lymph nodes; n.d., not done

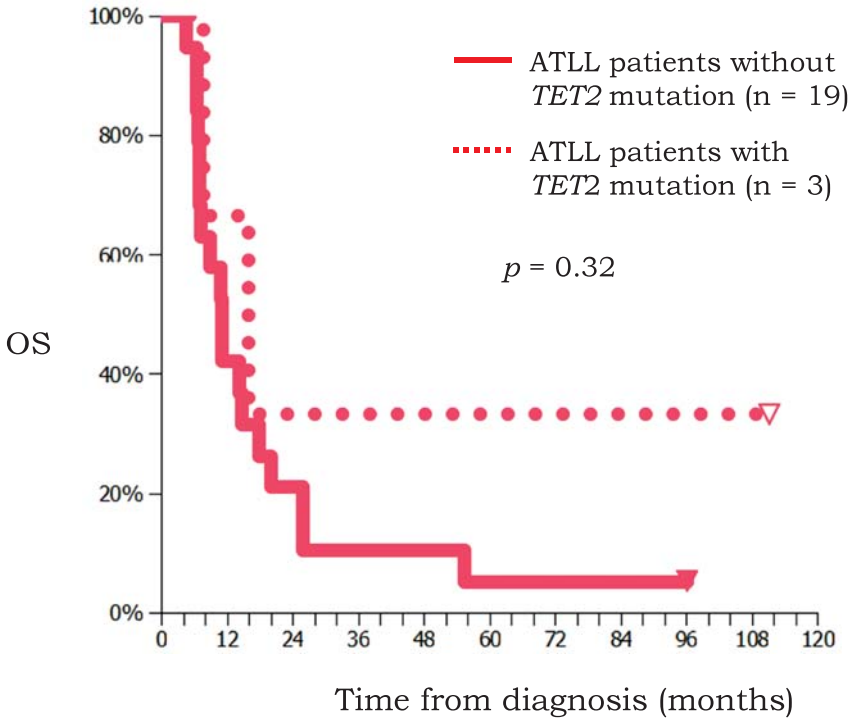

Fig. 1. Overall survival (OS) of ATLL patients with or without *TET2* mutation. ATLL, adult T-cell leukemia/lymphoma; TET2, ten-eleven translocation-2

and $CD4^{-}CD8^{-}$ in 1 case. The remaining 3 cases were characterized as follows: in 1 case, both CD4 and CD8 were weakly positive by immunohistochemistry; in 1 case, CD4 was positive but the status of CD8 was undetermined; and in 1 case, the status of neither CD4 nor CD8 was examined. In 9 patients, the proportion of infiltrated ATLL cells in each sample was analyzed by pathological examination. Except for case number 6, ATLL cells constituted more than half of the cells in the sample.

We examined the entire coding sequences of the *TET2* gene (exons 3-10) in 22 ATLL patients, and found a silent mutation in 1 patient (case 3) and missense mutations in 3 patients (cases 12, 14, and 20). In addition, 6 types of single nucleotide polymorphisms, as determined from referencing the base sequence in the Ensembl genome database (http://www.ensembl.org/Homo_sapiens/Transcript/Sequence_cDNA?db=core;g=ENSG00000162434;r=1:65071494-65204775;t=ENST00000342505), were identified in 15 cases, including 3 patients with mutations (cases 3, 14, and 20).

The missense mutations observed in cases 12, 14, and 20 were c.2440 C > T, c.2604 T > G, and c.3722 C > A, respectively, which led to amino acid substitutions from p.814 arginine (R) to cysteine (C), p.868 phenylalanine (F) to leucine (L), and p.1241 alanine (A) to aspartic acid (D), respectively. SIFT and PolyPhen-2 were used to predict the possible effects of these missense mutations on TET2. The SIFT scores of R814C, F868L, and A1241D were 0, 0.01, and 0, respectively, suggesting that these 3 missense mutations were deleterious. Furthermore, the PolyPhen-2 scores of R814C, F868L, and A1241D were 0.006, 0.169, and 0.971, respectively, indicating that these mutations were benign, possibly damaging, and probably damaging, respectively.

As of the end-of October 2015, two patients remained alive, one with a *TET2* missense mutation and the other without *TET2* mutation. The overall survival of ATLL patients with or without *TET2* mutation is shown in Fig. 1.

DISCUSSION

We identified three *TET2* missense mutations, R814C, F868L, and A1241D, in three different patients in our study population of 22 ATLL cases. Independent of this study, we recently performed a genome-wide analysis of ATLL cells and observed *TET2* mutation in approximately 10% of ATLL patients.[16] In these two independent ATLL cohorts, *TET2* mutation was observed with approximately the same frequency.

TET2 missense mutations have been reported to occur throughout the gene.[22] In particular, A1241D is located in a cysteine-rich domain that is the most frequently mutated region of *TET2* in myeloid disorders[22] and diffuse large B-cell lymphomas.[15] The mutations identified in this study were

evaluated using *in silico* analysis methods for predicting the mutational effects on protein function, and 2 of the mutations were determined to be damaging to protein function by both *in silico* methods. Because TET2 catalyzes the conversion of 5-methyl-cytosine to 5-hmC, deleterious mutations would affect the global methylation status of genes. In fact, *TET2* mutations are associated with hypermethylation within CpG islands, and at CpG-rich promoters of genes involved in hema topoietic differentiation and cellular development in diffuse large B-cell lymphoma. Furthermore, 11% of the hypermeth ylated genes, which include several tumor suppressor genes, were reported to be down-regulated. In ATLL, epigenetic changes to cell cycle regulator genes such as p53, p15, p14, and p16 have been reported,[23,24] which may be due to TET2 mutation and its functional impairment.

We recently reported that TET2 loss-of-function played a dual role in MPN, as both a disease initiator and disease accelerator.[25] *TET2* mutation was reported to be involved in the ontogeny of lymphoid malignancies. Some patients with a *TET2*-mutated myeloid malignancy developed lymphoma, and the same *TET2* mutation was observed both in the primary myeloid malignancies and the secondary lymphoma, indicating that the two diseases have common ancestry. Furthermore, in angioimmunoblastic T-cell lymphoma, the same *TET2* mutation was identified within both lymphoma and lineage-$CD34^-CD38^+$ progenitors from G-CSF-mobilized peripheral blood, indicating that *TET2* mutation occurred at the stem/progenitor cell level and was an early event in lymphoma ontogeny. In addition, TET2 loss-of-function demonstrated altered T- and B-cell lineage development in a mouse model.[8] On the other hand, we reported that in MPN, TET2 loss-of-function led to acceleration of malignancy;[25] in this instance, *TET2* mutation was thought to be a late genetic event. We could not definitively conclude whether *TET2* mutation was an early genetic event or a late genetic event in the ATLL patients in this study, because we did not evaluate the *TET2* mutational status in the myeloid lineage cells or hematopoietic progenitor cells due to the unavailability of patients' bone marrow samples.

In conclusion, *TET2* mutation was identified in several ATLL patients, and was likely to be involved in their leukemogenesis. In this study, the number of patients with *TET2* mutation was too small to elucidate specific clinical features associated with *TET2* mutation in ATLL. Overall survival appeared approximately equal, regardless of the presence or absence of *TET2* mutation. The accumulation of mutational analyses in a large number of ATLL patients will clarify the impact of *TET2* mutations on the ATLL clinical course.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

REFERENCES

1 Watanabe T: Current status of HTLV-1 infection. Int J Hematol 94:430-434, 2011
2 Yasunaga J, Matsuoka M: Leukaemogenic mechanism of human T-cell leukaemia virus type I. Rev Med Virol 17:301-311, 2007
3 Yasunaga J, Matsuoka M: Molecular mechanisms of HTLV-1 infection and pathogenesis. Int J Hematol 94:435-442, 2011
4 Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, *et al*.: Mutation in TET2 in myeloid cancers. N Engl J Med 360: 2289-2301, 2009
5 Langemeijer SM, Kuiper RP, Berends M, Knops R, Aslanyan MG, *et al*.: Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet 41:838-842, 2009
6 Koh KP, Yabuuchi A, Rao S, Huang Y, Cunniff K, *et al*.: Tet1 and Tet2 regulate 5-hydroxymethylcytosine production and cell lineage specification in mouse embryonic stem cells. Cell Stem Cell 8:200-213, 2011
7 Shide K, Kameda T, Shimoda H, Yamaji T, Abe H, *et al*.: TET2 is essential for survival and hematopoietic stem cell homeostasis. Leukemia 26:2216-2223, 2012
8 Quivoron C, Couronné L, Della Valle V, Lopez CK, Plo I, *et al*.: TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. Cancer Cell 20:25-38, 2011
9 Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, *et al*.: Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell 20:11-24, 2011
10 Li Z, Cai X, Cai CL, Wang J, Zhang W, *et al*.: Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. Blood 118: 4509-4518, 2011
11 Couronné L, Bastard C, Bernard OA: TET2 and DNMT3A mutations in human T-cell lymphoma. N Engl J Med 366:95-96, 2012
12 Lemonnier F, Couronné L, Parrens M, Jaïs JP, Travert M, *et al*.: Recurrent TET2 mutations in peripheral T-cell lymphomas correlate with TFH-like features and adverse clinical parameters. Blood 120:1466-1469, 2012
13 Odejide O, Weigert O, Lane AA, Toscano D, Lunning MA, *et al*.: A targeted mutational landscape of angioimmunoblastic T-cell lymphoma. Blood 123:1293-1296, 2014
14 Sakata-Yanagimoto M, Enami T, Yoshida K, Shiraishi Y, Ishii R, *et al*.: Somatic RHOA mutation in angioimmunoblastic T cell lymphoma. Nat Genet 46:171-175, 2014
15 Asmar F, Punj V, Christensen J, Pedersen MT, Pedersen A, *et al*.: Genome-wide profiling identifies a DNA methylation signature that associates with TET2 mutations in diffuse large B-cell lymphoma. Haematologica 98:1912-1920, 2013
16 Kataoka K, Nagata Y, Kitanaka A, Shiraishi Y, Shimamura T, *et al*.: Integrated molecular analysis of adult T cell leukemia/lymphoma. Nat Genet 47:1304-1315, 2015
17 Thiel A, Beier M, Ingenhag D, Servan K, Hein M, *et al*.:

Comprehensive array CGH of normal karyotype myelodysplastic syndromes reveals hidden recurrent and individual genomic copy number alterations with prognostic relevance. Leukemia 25:387-399, 2011

18 Kameda T, Shide K, Shimoda HK, Hidaka T, Kubuki Y, *et al*.: Absence of gain-of-function JAK1 and JAK3 mutations in adult T cell leukemia/lymphoma. Int J Hematol 92:320-325, 2010

19 Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, *et al*. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res 40:W452-457, 2012

20 Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, *et al*.: A method and server for predicting damaging missense mutations. Nat Methods 7:248-249, 2010

21 Shimoyama M: Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma. A report from the Lymphoma Study Group (1984-87). Br J Haematol 79:428-437, 1991

22 Mohr F, Dohner K, Buske C, Rawat VP: TET genes: new players in DNA demethylation and important determinants for stemness. Exp Hematol 39:272-281, 2011

23 Hofmann WK, Tsukasaki K, Takeuchi N, Takeuchi S, Koeffler HP: Methylation analysis of cell cycle control genes in adult T-cell leukemia/lymphoma. Leuk Lymphoma 42:1107-1109, 2001

24 Yamada Y, Kamihira S: Inactivation of tumor suppressor genes and the progression of adult T-cell leukemia-lymphoma. Leuk Lymphoma 46:1553-1559, 2005

25 Kameda T, Shide K, Yamaji T, Kamiunten A, Sekine M, *et al*.: Loss of TET2 has dual roles in murine myeloproliferative neoplasms: disease sustainer and disease accelerator. Blood 125:304-315, 2015