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Original Article

TET2 mutation in diffuse large B-cell lymphoma

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Ten-eleven translocation-2 (TET2) mutation is frequently observed in myeloid malignancies, and loss-of-function of TET2 is essential for the initiation of malignant hematopoiesis. *TET2* mutation presents across disease entities and was reported in lymphoid malignancies. We investigated *TET2* mutations in 27 diffuse large B-cell lymphoma (DLBCL) patients and found a frameshift mutation in 1 case (3.7%). *TET2* mutation occurred in some populations of DLBCL patients and was likely involved in the pathogenesis of their malignancies. 〔*J Clin Exp Hematop 56(3):145-149, 2017*〕

Keywords: TET2, epigenetic modifier, DLBCL

INTRODUCTION

Diffuse large B cell lymphoma (DLBCL) is an aggressive B cell neoplasm. Gene expression profiling divided DLBCL into the following two subgroups, whose cell origins were thought to differ[1]: germinal center B-cell (GCB) DLBCL and activated B-cell (ABC) DLBCL. Mutations in epigenetic modifiers, such as *MLL2*, *EZH2*, *CREBBP*, and *EP300*, were frequently observed in GCB DLBCL[2], whereas gene mutations that activated nuclear factor κB (NF-κB) signaling, such as mutations in *A20*, *CARD11*, *CD79B*, and *MYD88*, were frequently observed in ABC DLBCL[3, 4].

Epigenetic modifiers include histone-modifying enzymes and regulators of DNA methylation. Ten-Eleven Translocation-2 (TET2) is a regulator of DNA methylation, and plays a key role in the conversion of 5-methyl-cytosine (5-mC) to 5-hydroxymethyl cytosine (5-hmC)[5]. *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, 7]. Somatic mutations in *TET2* were first identified in myeloproliferative neoplasms (MPN) and myelodysplastic syndromes[8, 9]. In addition to myeloid malignancies, *TET2* mutations were detected in T and B lymphomas. Of these, *TET2* was most frequently mutated in angioimmunoblastic T-cell lymphomas (up to 76%) and "Th follicular (T_{FH})−like" peripheral T-cell lymphomas (PTCL), not otherwise specified[10-12] (19–51%). *TET2* mutation was also observed in approximately 10% of adult T cell leukemia/lymphoma cases[13, 14]. As for B-cell malignancies, 0–12% of DLBCL patients were reported to carry *TET2* mutation[15-18]. In this report, we examined the *TET2* mutation in 27 DLBCL patients.

MATERIALS AND METHODS
PATIENTS AND TUMOR SAMPLES

A series of 27 DLBCL patients with available frozen tumor cell samples was selected. The specimens were collected between 2006 and 2011. Medical records were reviewed for clinical data. This study was approved by the Research Ethics Committee of University of Miyazaki, and conducted in accordance with the Helsinki Declaration of 1975 as revised in 2008.

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TET2 GENOTYPING

DNA was extracted from frozen cells using a standard protocol. The coding sequence of the *TET2* gene (exons 3 through 10) was amplified by the polymerase chain reaction (PCR) method with a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). The sequences of PCR primers for *TET2* were designed as described in a previous report[19], and these primers were purchased from Hokkaido System Science Co., Ltd. The 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 (SNPs) was determined by referencing the assembled sequence in the Ensembl genome database[20].

RESULTS

Subject characteristics are listed in Table 1. Of the 27 patients, 16 were men and 11 were women. The median age was 72 years (range, 34−81). According to the International Prognostic Index (IPI), 2 patients were classified as low risk, 4 as intermediate-1 risk, 5 as intermediate-II risk, and 15 as high risk. The surface markers of DLBCL cells were analyzed by immunohistochemistry. Based on the immunostaining of CD10, BCL6, and MUM1, 7 patients were classified as GCB DLBCL, and 9 as non-GCB DLBCL.

We examined the entire coding sequence of the *TET2* gene (exons 3−10) in 27 DLBCL patients, and found a frameshift mutation in 1 patient (case 16) (Figure 1). In addition, 5 types of SNPs, 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 found in 15 cases, including the 1 patient with a *TET2* mutation (case 16).

The frameshift mutation observed in case 16 was c.2057_2058delGAinsAGG, which led to premature termination p.R686KfsX7. This short form mutant TET2 lacks the cysteine-rich domain and double stranded b-helix (DSBH) 2OG-Fe(II)-dependent dioxygenase domain.

DISCUSSION

We found one *TET2* frameshift mutation in 27 DLBCL cases. This frameshift mutation led to premature termination p.R686KfsX7, and formed truncated-type TET2 that lacks the cysteine-rich domain and DSBH 2OG-Fe(II)-dependent dioxygenase domain. Reported somatic mutations in *TET2* in myeloid and lymphoid malignancies included missense, nonsense, and frameshift mutations, and thus, the TET2 mutation was thought to be loss-of-function mutation[21]. As TET2 catalyzes the conversion of 5-mC to 5-hmC, loss-of-function mutations would affect the global methylation status of genes[5]. Indeed, there were decreased 5-hmC levels in the DNA of myeloid malignancy patients with *TET2* mutation compared with those without *TET2* mutation or healthy controls[7]. We and others reported that *TET2*-deficient hematopoietic stem cells (HSCs) exhibited increased self-renewal ability and had a competitive growth advantage over wild-type HSCs[15, 22-24]. This augmented self-renewal activity in HSCs may be the basis for *TET2*-mutated myeloid malignancies. *TET2* mutation was also observed in a proportion of normal elderly individuals who exhibited clonal hematopoiesis, and one of seven such individuals subsequently developed MPN[25]. The situation should be the same with DLBCL. For the development of DLBCL, several gene mutations were required[26]. *TET2* mutation was one of them, and DLBCL may develop with additional gene mutations.

TET2 mutation was reported in 0–12% of DLBCL patients[15-18], and in our study one of 27 DLBCL patients (3.7%) carried a *TET2* mutation. As *TET2* mutation was frequently observed in AITL, we examined whether case 16 harbored composite lymphoma with DLBCL and AITL[27]. We carefully re-evaluated the biopsy sample from case 16, but could not find any morphological or immunohistochemical aspects of AITL. The lower incidence of *TET2* mutation in our DLBCL cohort compared with previous reports may be due to racial differences or differences in methodology to detect the mutation. We analyzed *TET2* mutation in Japanese patients, whereas previous reports analyzed Caucasian patients, and we detected the mutation by Sanger sequencing after PCR, whereas denaturing gradient gel electrophoresis was adopted to detect in the previous report[18].

Mutations in epigenetic modifiers were dominantly found in GCB DLBCL, but our case (case 16) was classified as non-GCB DLBCL. As the cell-of-origin subtypes were not identified in reported DLBCL with *TET2* mutations[15-18] and we found only one non-GCB DLBCL patient with a *TET2* mutation, we cannot conclude whether *TET2* mutations accumulated in GCB DLBCL, as is the case with *EZH2* mutation. Asmar et al. studied both a DNA methylation signature and the *TET2* mutation in DLBCL, and found that *TET2* mutation is associated with hyper-methylation within CpG islands, and at CpG-rich promoters of genes involved in hematopoietic differentiation and cellular development[18]. They also reported that 11% of the hyper-methylated genes, which include several tumor suppressor genes, were down-regulated. These epigenetic changes may be due to *TET2* mutation and its functional impairment, and may be involved in the ontogeny of DLBCL.

In conclusion, *TET2* mutation was observed in one of 27 DLBCL patients (3.7%), and was likely to be involved in DLBCL ontogeny.

Table 1. Profiles and clinical data for each DLBCL case

case	age	sex	PS	LDH	stage	IPI	samples	Phenotype of lymphoma cells in the sample				GCB / non-GCB
no.	y		ECOG	IU/L	(Ann Arbor)			CD20	CD10	BCL-6	Mum-1	
1	74	M	0	335	IV	high	LNs	+	n.d.	n.d.	n.d.	n.a.
2	62	F	1	882	IV	high	Tumor	+	n.d.	n.d.	n.d.	n.a.
3	34	M	1	600	II	int1	LNs	+	n.d.	n.d.	n.d.	n.a.
4	58	M	n.a.	n.a.	n.a.	n.a.	Bone	+	n.d.	n.d.	n.d.	n.a.
5	73	M	3	1437	IV	high	LNs	+	n.d.	-	+	n.a.
6	74	M	2	397	IV	high	Tumor	+	-	n.d.	focally +	non-GCB
7	64	F	0	268	I	int2	LNs	+	+	+	focally +	GCB
8	34	F	0	113	II	low	LNs	+	+	-	+	non-GCB
9	42	M	1	138	IV	int1	LNs	+	+	+	+	non-GCB
10	77	M	2	254	IV	high	Tumor	+	±	+	+	non-GCB
11	80	M	1	242	IV	high	Tumor	+	-	-	+	non-GCB
12	77	M	3	1256	IV	high	LNs	+	-	-	+	non-GCB
13	74	M	1	147	IV	high	LNs	+	-	-	+	n.a.
14	75	M	0	279	IV	high	LNs	+	-	+	+	GCB
15	67	F	0	242	III	high	LNs	+	-	n.d.	-	n.a.
16	68	F	2	326	IV	high	LNs	+	-	-	+	non-GCB
17	70	M	4	517	IV	high	Adrenal gland	+	-	-	+	non-GCB
18	79	F	4	234	IV	high	Tumor	+	+	focally +	focally +	GCB
19	60	F	4	249	III	high	LNs	+	±	+	+	n.a.
20	34	F	1	743	IV	int2	Bone	+	-	+	±	GCB
21	81	M	1	210	II	int1	Tumor	+	-	+	+	n.a.
22	72	M	2	532	IV	high	Tumor	+	-	+	n.d	n.a.
23	79	F	1	192	IV	int2	Tumor	+	-	-	focally +	GCB
24	53	F	1	389	III	int2	Bone	+	±	±	+	non-GCB
25	73	M	1	179	III	int2	LNs	+	+	+	+	GCB
26	52	M	0	219	II	low	LNs	+	-	+	-	GCB
27	80	F	0	176	I	int1	Tumor	+	-	-	focally +	n.a.

The results of immunohistochemical staining of tumor samples are shown as +, ±, or -, corresponding to positive, weak positive, or negative, respectively. n.a.: data not available, n.d.: not done

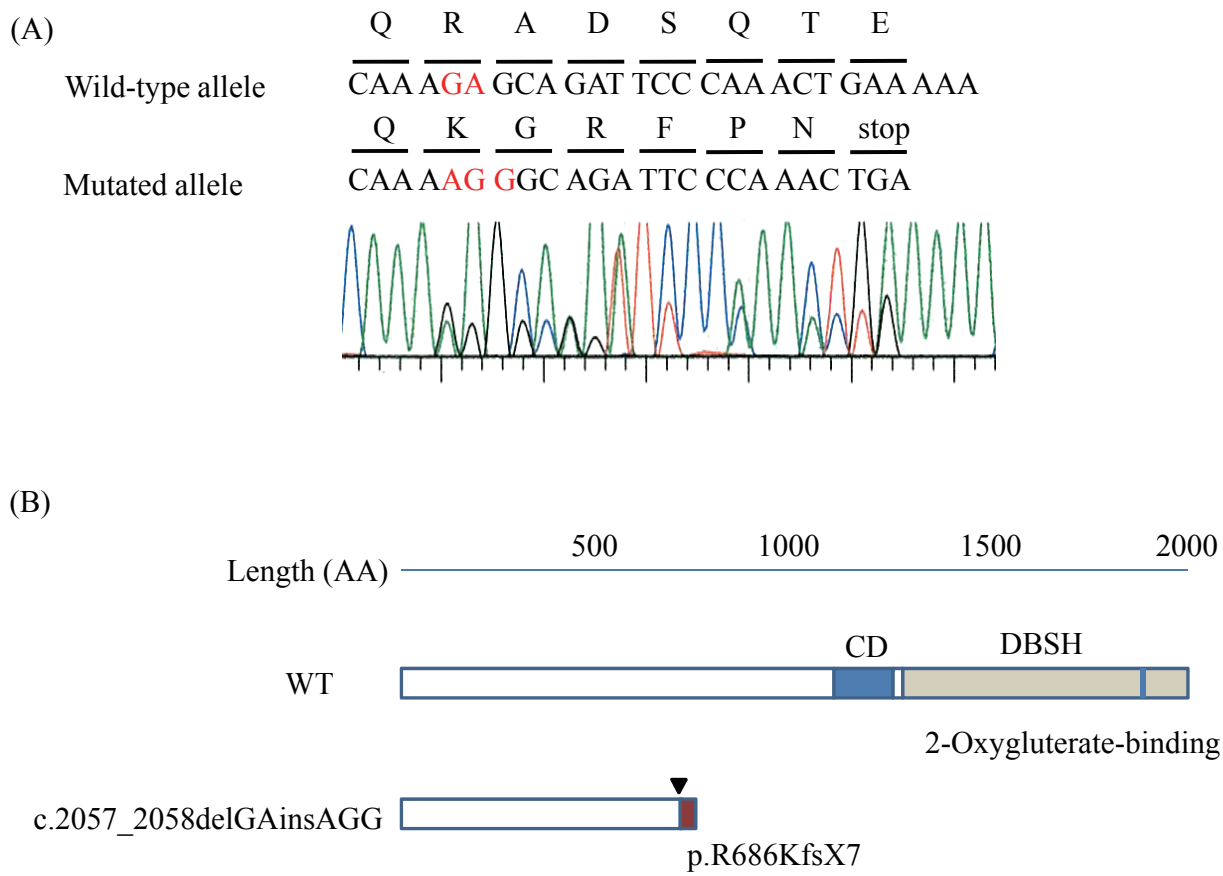

Figure 1. *TET2* mutation in DLBCL and TET2 protein
A. Sanger sequence of *TET2* in case 16. Electropherogram of *TET2* exon3 sequences showing the monoallelic mutation c.2057_2058delGAinsAGG (note in red). The DNA and corresponding amino acid sequences of the wild-type and mutant TET2 alleles are also shown.
B. A schematic representation of the TET2 protein. The arrowhead shows the position corresponding to the mutation. This mutation led to premature termination p.R686KfsX7. Truncated-form mutant TET2 lacks the cysteine-rich domain (CD) and double stranded b-helix (DSBH) 2OG-Fe(II)-dependent dioxygenase domain.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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