Duodenal and nodal follicular lymphomas are distinct: the former lacks activation-induced cytidine deaminase and follicular dendritic cells despite ongoing somatic hypermutations

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Although most follicular lymphomas are believed to be of nodal origin, they sometimes originate from the duodenum. We have reported that the latter differ from nodal follicular lymphomas in having lower clinical stages and uniformly low histological grades, along with variable region of immunoglobulin heavy chain gene (VH) usage that is more similar to mucosa-associated lymphoid tissue (MALT) lymphomas. Little is known, however, about whether they possess other characteristics of nodal follicular lymphomas, particularly ongoing mutations with follicular dendritic cells. We examined 17 cases for which PCR identified the monoclonal bands of the immunoglobulin gene. The duodenal cases showed ongoing mutations, but they lacked activation-induced cytidine deaminase (AID) expression, a statistically significant difference from the nodal cases ($P < 0.001$), and their follicular dendritic cell networks were disrupted. Moreover, not only were VH deviations observed but also they used very restricted VH genes. Although the mechanisms of ongoing mutation without AID and follicular dendritic cell were not clarified, restricted VH usage strongly suggested that antigen stimulation was involved, and that was similar to MALT lymphomas. In conclusion, duodenal follicular lymphomas were shown to be unique, in that they had ongoing hypermutations such as nodal cases, but the mechanisms involved in the hypermutation were quite different; furthermore, restricted VH usage suggested a strong similarity to the antigen-dependent origin of MALT lymphomas.

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We have reported that duodenal follicular lymphomas are frequently found in the second portion of the duodenum.¹ Although they express CD10 and bcl-2 and harbor t(14;18) translocation similar to nodal follicular lymphomas,² most of them are obviously at lower clinical stages than the nodal cases (most are stage I–II) and have lower histolo-

gical grading with rather dominantly uniform intermediate small cleaved cells.

The vast majority of follicular lymphomas originate from lymph nodes, and earlier reports have clearly indicated that these follicular lymphoma cells derive from germinal-center B cells: both cells share characteristics such as accumulation of somatic hypermutation and ongoing mutations.³ Furthermore, they interact with follicular dendritic cells. In an earlier report, we also described that duodenal follicular lymphomas have a high frequency of IgVH4 gene usage as in mucosa-associated lymphoid tissue (MALT) lymphomas, with which they share other characteristics.^{4–6} Localized disease is one of main characteristics of MALT lymphomas.

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From these findings, we considered whether duodenal follicular lymphomas have other differences from nodal follicular lymphomas, and decided to examine their expression of activation-induced cytidine deaminase (AID), which plays the important roles of class-switch recombination and somatic hypermutation,⁷ and the patterning of their follicular dendritic cell networks. It is reported that AID expression is associated with ongoing mutation in nodal follicular lymphoma,⁸ but is not correlated with diffuse large B-cell lymphomas.⁹ Although other characteristics of duodenal follicular lymphomas have been described, to the best of our knowledge no report has focused on their somatic and ongoing mutations, which are the most important similarity to germinal-center B-cell lymphomas.

In this report, we discovered that duodenal follicular lymphomas showed ongoing somatic hypermutations similar to nodal cases; unlike the latter, however, they lack AID and have disrupted follicular dendritic cell networks.

Materials and methods

Patient Selection

Buffered formalin-fixed and paraffin-embedded tissues (10%) were used for histological, immunohistochemical and immunogenotypical studies. We chose 30 consecutive patients of duodenal follicular lymphoma, successfully amplified the monoclonal rearranged band of the *VH* gene in 17 of these patient samples, and focused on these samples for further analyses. Informed consent for examination was obtained for the use of all samples.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections were subjected to immunohistochemical staining. Staining was carried out using heat-induced epitope retrieval or trypsin-induced retrieval, an avidin-biotin complex method, and an automated immunostainer (Ventana Medical System, Tuscon, AZ, USA), as described earlier.¹⁰ The antibody panel used to assess these cases was as follows (clone, dilutions): CD20 (L26, 1:200), CD3 (PS-1, 1:50), CD10 (56C6, 1:50), CD5(4C7, 1:100), bcl-2 (3.1, 1:200), CD23 (1B12, 1:100) and Ki-67 (MIB-1, 1:5000) (Novocastra, Newcastle-upon-Tyne, UK); CD21 (1F8, 1:20), MUM1 (MUM1p, 1:50) (DAKO Cytomation, Glostrup, Denmark A/S); bcl-6 (D-8, 1:100) (Santa Cruz, CA, USA); and cyclin D1 (SP4, ready to use) (Nichirei, Tokyo, Japan); AID (ZA001, 1:100) (ZYMED, South San Francisco, CA, USA). As for CD20, CD3, CD10, CD5, cyclin D1, bcl-2, bcl-6 and MUM-1 antigens, positivity was determined when 30% or more lymphoma cells were positive for their antibodies. For AID expression in tumor

follicles, samples with 20% or more expressing cells were evaluated as positive. Ki-67-positive cells were counted in tumor follicles.

DNA Extraction and PCR

DNA was extracted from paraffin-embedded tissue using the QIAamp DNA Micro Kit- (Qiagen Inc., Valencia, CA, USA). The variable region (CDR2 and FW3) and *VDJ* region (CDR3) of the immunoglobulin heavy-chain gene were amplified by semi-nested PCR, using the primers of FR2, *LJH* and *VLJH* as described earlier.^{11,12} Primers were as follows: 5'-CC GGRAARRGTCTGGAGTGG-3', as upstream consensus *V* region primer (FR2); 5'-CTTACCTGAGGA GACGGTGACC-3', as a consensus *J* region primer (*LJH*); 5'-GTGACCAGGGTNCCTTGGCCCC-3', as a consensus *J* region primer (*VLJH*). PCR products were purified using the QIAquick PCR purification kit (Qiagen). Then 1 μ l of the PCR product was applied for direct sequencing (ABI PRISM Model 3100, version 3.7, Applied Biosystems).

Ongoing Mutation Study and Analysis

PCR products were ligated into the pDrive cloning vector and transformed into DH5alpha cells (TaKaRa Bio. Inc., Tokyo, Japan) according to the instruction manual (PCR cloning kit, Qiagen). After an overnight culture, 10 and more white colonies were picked from a Luria-Bertani (LB) agar plate based on the X-Gal screening, then placed into 25 μ l of the Insert Check ready kit solution (TOYOBO, Osaka, Japan). PCR conditions consisted of 30 cycles of 94 °C for 30 s, 60 °C for 5 s and 72 °C for 30 s. Ten samples including correct PCR products confirmed by checking 2% gel electrophoresis for each case were sequenced by the same method as described above. Then, the closest germline was searched for by BLAST using the immunoglobulin sequence (NCBI). Ongoing mutation was determined by dividing the cumulative number of partially shared mutations (mutations shared by some clones but not by all the *VH* gene clones) and unique mutations (mutations unique to a distinct *VH* gene clone) with the expected number of mutations calculated based on the PCR error rate (4.5×10^{-4} change per base per PCR cycle) by the method described above.⁷ The χ^2 test with Yates' correction when appropriate was used to evaluate the association of qualitative variables in the different groups. Statistical analysis was carried out using Statcel2 for Windows. Values of $P < 0.05$ were considered statistically significant.

Fluorescence *In Situ* Hybridization

Fluorescence *in situ* hybridization (FISH) for t(14;18)(q32;q21)/*IGH-BCL2* translocations were

carried out using the *BCL2* FISH DNA split signal probe (DAKO Cytomation, Glostrup, Denmark A/S) according to the manufacturer's instructions. We

examined FISH directly on paraffin-embedded tissue sections and detected split signal of *BCL2* gene as described earlier.¹³

Table 1 Clinicopathologic features of duodenal follicular lymphoma

Patient no.	Age/sex	Stage	Grade	CD5	CyclinD1	CD10	<i>bcl-2</i>	<i>bcl-6</i>	MUM-1	<i>t</i> (14;18)	CD21 pattern
1	75/M	I	1	—	—	+	+	+	—	Not tested	Duodenal
2	57/M	I	1	—	—	+	+	+	—	Not tested	Duodenal
3	58/M	IV	1	—	—	+	+	+	—	+	Duodenal
4	75/M	II2	1	—	—	+	+	+	—	—	Duodenal
5	71/F	I	1	—	—	+	+	+	—	+	Duodenal
6	66/F	I	1	—	—	+	+	+	—	—	Nodal
7	51/M	I	1	—	—	+	+	+	—	+	Duodenal
8	49/M	II2	1	—	—	+	+	+	—	+	Duodenal
9	62/F	II2	1	—	—	+	+	+	—	+	Duodenal
10	54/M	I	1	—	—	+	+	+	—	+	Duodenal
11	61/F	IV	1	—	—	+	+	+	—	+	Duodenal
12	53/F	II2	1	—	—	+	+	+	—	+	Duodenal
13	57/F	I	2	—	—	+	+	+	—	+	Duodenal
14	56/M	II2	1	—	—	+	+	+	—	+	Duodenal
15	66/F	II2	1	—	—	+	+	+	—	+	Duodenal
16	55/F	II2	1	—	—	+	+	+	—	+	Nodal
17	63/F	II2	1	—	—	+	+	+	—	+	Duodenal

F, female; M, male.

Immunophenotypic data were analyzed using immunohistochemistry.

Figure 1 Duodenal follicular lymphoma and immunohistochemical stain (patient no.17). (a) H&E: A neoplastic follicle is composed of uniform intermediate small-cleaved cells. (b) Tumor cells are positive for CD20 in all cases (c) and for CD10 (d) and *bcl-2*.

Results

Patient Summary and CD21 Expression

Clinical features (age, gender, clinical stage) and the results of immunohistochemical study are shown in Table 1. The 17 patients (8 males and 9 females) ranged in age from 49 to 75 years with a median age of 61 years. According to the International Workshop (Lugano) Classification, seven cases were clinical stage I, eight cases were stage II and two cases were stage IV.¹⁴ Histological grade were 1–2 of all cases. All patient samples expressed CD20, CD10, bcl-2 and bcl-6, and were negative for CD5, MUM1 and cyclin D1 by immunohistochemical study (Figure 1). In all cases, the Ki-67 labeling index was <10%. The presence of a t(14;18)(q32;q21)/IGH-BCL2 was sought by FISH depending on the material available. The translocation of 18q21 was detected in 13 of 15 (86.7%) cases (Figure 2 shows the split signal of patient no. 7 sample). We could not detect translocations at two patients' materials.

A typical pattern of follicular dendritic cells expressing CD21 and CD23 in nodal follicular lymphoma is shown in Figure 3 and clearly indicates neoplastic follicles. Nodal follicular lymphoma interacts with follicular dendritic cells, which sometimes provides a diagnostic clue.¹⁵ On the contrary, out of 17 samples of duodenal follicular lymphomas, 15 showed a very similar pattern of follicular dendritic cells: they were rather densely arranged at the periphery of the neoplastic follicle area but few follicular dendritic cells were detected at the center of the neoplastic follicle area.

In most nodal follicular lymphomas, follicular dendritic cell networks occupied more than two-thirds of the neoplastic follicle areas. On the contrary, follicular dendritic cell networks occupied <10% of the neoplastic follicle areas in duodenal follicular lymphomas, and were distributed in the periphery of the neoplastic follicles. Accordingly, the nodal pattern was identified as neoplastic follicles coextensive with large follicular dendritic cell networks, whereas the duodenal pattern was

Figure 2 Expression of AID and FISH. (a) AID expression in nodal follicular lymphoma, grade 1. More than 20% of neoplastic cells are positive. (b) AID expression in duodenal follicular lymphoma (patient no. 8). Almost all neoplastic cells are negative. (c) AID expression in patient no. 2. Some positive cells are shown (arrows). (d) BCL2 split signal of patient no. 7 (arrows: The FISH DNA probes are a mixture of a Texas Red-labeled DNA probe (bcl-2-upstream) and a fluorescein-labeled DNA probe (bcl-2-downstream). Split signals of red- and green-labeled probe are seen in the lymphoma cells, indicating breaking apart of the bcl-2 gene.

Figure 3 Expression of bcl-6, CD21 and CD23. (a) Tumor cells are positive for bcl-6 in all cases (shows patient no. 8). (b) CD21 expression in nodal follicular lymphoma, grade 1. Follicular dendritic cells are densely found in neoplastic follicles. (c) CD23 expression in duodenal follicular lymphoma (patient no. 17). Follicular dendritic cells are arranged at the periphery of the neoplastic follicle. (d) CD21 expression in other case of duodenal follicular lymphoma. Similar to CD23 expression.

identified as neoplastic follicles with disrupted, peripheral follicular dendritic cell networks.

AID Expression

In the duodenal follicular lymphomas, one patient sample (no. 9) expressed AID but other samples (16 samples) were almost completely negative for it (Figure 2). By contrast, out of 19 nodal follicular lymphomas, which histological grades were 1–2 in all cases, AID was clearly detected in 16 (Figure 2, data not shown). The difference in AID expression between nodal follicular lymphoma and duodenal follicular lymphoma was significant ($P < 0.001$).

Relationship among Follicular Dendritic Cells, AID, VH and Ongoing Mutation

The VH gene usage and analysis of somatic hypermutation are shown in Table 2. We sequenced 17 cases of duodenal follicular lymphoma. The VH gene usage included 9 (53%) samples of VH3, 5

(29%) samples of VH4 and 3 (18%) samples of VH5. The distribution of mutations in CDR2 and FR3 are also shown in Table 2. Regardless of AID expression, all samples displayed somatic hypermutation; the mutation frequency was 0.7–15.6%, with an average of 9.1%. In VH5 usage cases, there tended to be a low mutation frequency (0.7–8.8%, average 3.8%).

In Table 3, most samples showed ongoing mutations, yielding an average of 19.6-fold more mutations than the expected number of additional mutations because of PCR error. Two AID-negative samples (no. 8 and no. 12) did not have any partially shared or unique mutations. Table 4 shows the nucleotide sequence of the VH gene of patient nos. 12 and 7. In no. 7, there were four partially shared mutation and five unique mutations.

Discussion

Follicular lymphoma is one of the most frequent indolent lymphomas, and most of them are of nodal

Table 2 AID expression and immunoglobulin gene analysis

Patient no.	AID expression ^a	VH usage	Number of bases analyzed	CDR2		FR3		Total		Mutation rate (%) ^b
				R	S	R	S	R	S	
1	—	VH3-73	153	6	1	7	2	15	3	11.8
2	—	VH3-48	147	3	1	1	3	4	4	5.4
3	—	VH3-72	153	7	0	5	4	12	4	10.5
4	—	VH4-34	140	5	0	7	2	12	2	10
5	—	VH5-a	147	0	0	1	0	1	0	0.7
6	—	VH3-73	141 ^c	6	0	6	1	12	1	9.3
7	—	VH4-b	144	7	0	7	2	14	2	11.1
8	—	VH4-39	144	4	0	6	4	10	4	9.7
9	+	VH3-15	153	8	0	2	6	10	6	10.5
10	—	VH5-51	147	2	0	1	0	3	0	2
11	—	VH5-51	147	3	2	5	3	8	5	8.8
12	—	VH4-61	144	2	1	2	1	4	2	4.2
13	—	VH4-39	144	6	1	3	3	9	4	9
14	—	VH3-73	153	7	2	3	3	10	5	9.8
15	—	VH3-23	147	12	0	8	3	20	3	15.6
16	—	VH3-23	147	6	3	5	3	11	6	11.6
17	—	VH3-23	147	9	2	8	2	17	4	14.3

CDR, complementary determining region; FR, framework region; R, replacement mutation; S, silent mutation.

^aImmunohistochemically positive >20% in tumor follicles.^bDividing the total number of R and S mutations in CDR2 and FR3 by the number of bases analyzed.^cIn patient no. 6, deletion of nine nucleotides were found.**Table 3** AID expression and ongoing mutation in duodenal follicular lymphoma

Patient no.	AID expression	Number of clones analyzed	Number of bases sequenced	Point mutations				Number of mutations expected by PCR error	Ongoing mutation
				Total	Shared	Partially shared	Unique		
1	—	10	153	23	16	1	6	0.31	22.6
2	—	10	147	10	7	1	2	0.135	22.2
3	—	9	153	18	15	2	1	0.24	12.5
4	—	10	140	18	14	0	4	0.22	18.2
5	—	10	147	6	1	0	5	0.081	61.7
6	—	10	141	21	14	4	3	0.28	25
7	—	8	144	21	12	4	5	0.28	32.1
8	—	10	144	15	15	0	0	0.2	0
9	+	10	153	18	17	0	1	0.24	4.2
10	—	8	147	6	3	0	3	0.081	37
11	—	7	147	14	13	0	1	0.19	5.3
12	—	9	144	6	6	0	0	0.081	0
13	—	9	144	19	14	0	5	0.26	19.2
14	—	10	153	19	15	0	4	0.26	15.4
15	—	10	147	31	23	8	8	0.42	38.1
16	—	10	147	22	19	0	3	0.3	10
17	—	10	147	24	21	1	2	0.32	9.4

origin. But recently, intestinal (especially duodenal) follicular lymphomas have appeared with more frequency, and these are considered variants of follicular lymphoma in the fourth WHO classification.¹⁶ Interestingly, from our surgical files, only 5.8% (17/290 cases) of follicular lymphomas were extranodal up to 2001, whereas 30.8% (153/497 cases) were extranodal in 2002–2007. This frequency needs to be investigated hereafter, but consultation from other institution to our

department has not changed in the past 10 years, hence this frequency indicates that intestinal follicular lymphomas have become popular and the incidence of finding them is increasing. The similarity of duodenal follicular lymphomas to MALT lymphomas has been reported from different points of view: expression of $\alpha 4\beta 7$, which is a mucosal homing receptor,¹⁷ VH gene deviation,⁶ and IgA production suggesting that lymphoma cells derive from mucosal B cells.¹⁸

Table 4 Cloning assay of the VH gene of duodenal follicular lymphoma

CDR2		FR3
Patient no. 12		
VH4-61*08	TATATCTATTACAGTGGGAGCACCAACTACAACCCCTCCCTCAAGAGT	CGAGTCACCATATCAGTAGACACGTCCTCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGA
No. 12	-----C-----G-----T-----	-----C-----A-----C-----
Clone 01	-----C-----G-----T-----	-----C-----A-----C-----
Clone 02	-----C-----G-----T-----	-----C-----A-----C-----
Clone 03	-----C-----G-----T-----	-----C-----A-----C-----
Clone 04	-----C-----G-----T-----	-----C-----A-----C-----
Clone 05	-----C-----G-----T-----	-----C-----A-----C-----
Clone 06	-----C-----G-----T-----	-----C-----A-----C-----
Clone 07	-----C-----G-----T-----	-----C-----A-----C-----
Clone 08	-----C-----G-----T-----	-----C-----A-----C-----
Clone 09	-----C-----G-----T-----	-----C-----A-----C-----
Patient no. 7		
VH4-b*02	AGTATCTATCATAGTGGGAGCACCTACTACAACCCGTCCTCAAGAGT	CGAGTCACCATATCAGTAGACACGTCCTCAAGAGCCAATTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCAGACACGGCCGTGTATTACTGTGCGAGA
No. 7	-----C-CA-----C-----GT--G-----CT--	-----C-----GT-----G-A--C--C-----A-----
Clone 01	--A--C-CA-----C-----GT-----CT--	-----C-----GT-----A-----G-A--C--C-----A-----
Clone 02	-----C-CA-----C-----GT-----CT--	-----C-----GT-----G-A--C--C-----A-----
Clone 03	-----C-CA-----C-----GT-----CT--	-----C-----GT-----G-A--C--C-----A-----
Clone 04	-----C-CA-----C-----T-----CT--	-----C-----GT-----G-A--C--C-----A-----
Clone 05	-----C-CA-----C-----GT-----CT--	-----C-----GT-----G-A--C--C-----A-----
Clone 06	-----C-CA-----C-----GT-----CT--	-----C-----GT-----G-A--C--C-----A-----
Clone 07	-----C-CA-----C-----GT--G-----CT--	-----C-----T-----G-A--C--C-----A-----
Clone 08	-----C-CA-----C-----GT--G-----CT--	-----C-----GT-----G-A--C--C-----A-----

According to our earlier data, we analyzed eight samples for VH usage, and in this study, we analyzed the other 17 samples for VH usage. Therefore, in the 25 samples, 13 (52%) were VH3, 8 (32%) were VH4, and 4 (16%) were VH5. Phenotypically, IgM+ memory B cells resemble marginal zone B cells and are thought to be their circulating counterparts.¹⁹ Tsuiji *et al*²⁰ reported IgM+ memory B cells more frequently express VH3 family genes than do naive B cells. Noppe *et al*²¹ reported VH usage in nodal follicular lymphoma cases as VH3 70%, VH4 19% and VH1 11%; Bahler *et al*²² reported it as VH3 67%, VH4 22% and VH1 8%. From their data, duodenal follicular lymphomas of our series showed a higher usage of VH4 and VH5 than did nodal cases. Moreover, VH4-34 and VH5-51 were detected in three samples each in our study. This must not be coincidental, and selective usage strongly suggests that some antigen-dependent mechanism is involved in tumor development, as in the case of MALT lymphoma, which develops from chronic inflammation such as *Helicobacter pylori*-related gastritis.

CD21 is a complement receptor expressed in B cells and follicular dendritic cells in humans.²³ In nodal follicular lymphoma, follicular dendritic cells were important to the microenvironment in which follicular lymphoma cells grow.²⁴ As shown in the results, although follicular dendritic cell networks were located at the periphery of neoplastic follicles, the major center part of follicular dendritic cells looked broken except for in two samples; by contrast, all nodal lymphomas showed extensively distributed follicular dendritic cells. The shape of degraded follicular dendritic cells are somewhat similar to the follicular colonization of MALT lymphomas, in which marginal zone B-cell lymphoma cells penetrate the non-neoplastic germinal centers and destroy follicular dendritic cell networks (Figure 4). Patient nos. 6 and 16 of the duodenal series showed nodal-pattern follicular dendritic cell. Interestingly, patient no. 16 was associated with markedly swollen mesenteric lymph nodes. Therefore, duodenal lesions of this case were prominent, but the primary site of this case was not clearly determined. Patient nos. 3 and 11 were clinical stage IV, but showed duodenal-pattern follicular dendritic cell. We believe that these duodenal lymphomas possibly progressed to nodal lymphomas.

AID plays key roles in class switching and somatic hypermutation of heavy chains in germinal-center B cells.⁷ In an earlier report, RT-PCR showed that follicular lymphoma cells are positive for AID.⁸ We examined the samples immunohistochemically, and found that nodal follicular lymphomas had 20% or more AID-positive cells, in contrast to only a few (<1%) duodenal follicular lymphoma cells that were AID-positive. Our data indicate that most duodenal follicular lymphomas have somatic and ongoing mutations in spite of the lack of AID

Figure 4 CD21 expression in reactive lymphoid hyperplasia of the duodenum and in MALT lymphoma of the stomach. (a) CD21 expression in reactive lymphoid hyperplasia of the duodenum. Follicular dendritic cell network are present. (b) CD21 expression in MALT lymphoma of the stomach. Follicular dendritic cell network are disrupted (follicular colonization), which is similar to those of duodenal follicular lymphomas. (c) CD21 expression in a non-neoplastic germinal center of a gastric MALT lymphoma. The follicular dendritic cell networks are well preserved.

expression. This sounds contradictory; however, Bombardieri *et al*²⁵ reported that neoplastic marginal zone-like B cells are AID-negative but have somatic and ongoing mutations.²⁶ On the other hand, Smit *et al*,²⁷ Pasqualucci *et al*²⁸ and Lossos *et al*⁹ reported of lack of correlation between AID expression and intraclonal diversity of *VH* genes in B-cell non-Hodgkin's lymphomas. And Smit *et al*²⁷ mentioned that AID expression at follicular lymphomas were 25%, and intraclonal variation was found in the absence of AID, but they did not explained histology, immunophenotype and stage. *IgVH* mutations were introduced at a tumor stage when AID enzyme was still expressed. However, from our data, almost all of duodenal follicular lymphoma lacks AID in the protein expression level, and histological grade was 1–2. Moreover, all samples expressed *bcl-6*, which is known to be a target gene for somatic hypermutation in germinal-center B cells.²⁹ The mechanisms of somatic

and ongoing mutation are not clear. Therefore, in duodenal follicular lymphomas, somatic and ongoing mutation may be independent of AID expression.

Conclusions from our data are as follows: duodenal follicular lymphoma cells showed somatic and ongoing mutations similar to nodal ones, as well as CD10 and *bcl-2* expressions; however, they did not express AID, and the follicular dendritic cell networks essential for 'follicular pattern' lymphomas were severely disrupted, as in the follicular colonization of MALT lymphomas. We also found out that duodenal follicular lymphoma had *VH* family deviation marked by the presence of very restricted *VH4* and *VH5* segments, which strongly indicated that the lymphoma cells are derived from B cells reacting with specific antigens. These findings strongly suggested that duodenal follicular lymphoma is distinct from nodal follicular lymphoma, and has more similarity to MALT chronic

inflammation-based lymphomas; these similarities will be very important in considering therapeutic countermeasures against this disease.

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Conflict of interest

The authors declare no conflict of interest.

Authorship statement

Takata K, Sato Y, Nakamura N and Yoshino T designed and performed the research. Takata K, Okada H and Tari A prepared specimens and clinical data. Takata K, Kikuti YY, Ichimura K, Tanaka T, Morito T, Tamura M, Oka T and Kondo E analyzed the data. Takata K wrote the paper.

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