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CD10 down expression in follicular lymphoma correlates with gastrointestinal lesion involving the stomach and large intestine

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Key words

CD10, downexpression, follicular lymphoma, gastrointestinal tract, involvement of stomach and large intestine

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Follicular lymphoma (FL) shows co-expression of B-cell lymphoma 2 (BCL2) and CD10, whereas downexpression of CD10 is occasionally experienced in gastrointestinal (GI) FL with unknown significance. Gastrointestinal FL is a rare variant of FL, and its similarity with mucosa-associated lymphoid tissue lymphoma was reported. We investigated the clinicopathological and genetic features of CD10 downexpressed ($CD10^{down}$) GI-FL. The diagnosis of $CD10^{down}$ FL was carried out with a combination of pathological and molecular analyses. The incidence of $CD10^{down}$ GI-FL was shown in 35/172 (20.3%) cases, which was more frequent than nodal FL (3.5%, $P < 0.001$). The difference was additionally significant between GI-FL and nodal FL when the analysis was confined to primary GI-FL (55.2% *vs* 3.5%, $P < 0.001$). Compared to $CD10^{+}$ GI-FL, $CD10^{down}$ GI-FL significantly involved the stomach or large intestine ($P = 0.015$), and additionally showed the downexpression of BCL6 ($P < 0.001$). The follicular dendritic cell meshwork often showed a duodenal pattern in the $CD10^{down}$ group ($P = 0.12$). Furthermore, a lymphoepithelial lesion was observed in 5/12 (40%) gastric FL cases, which indicated caution in the differentiation of mucosa-associated lymphoid tissue lymphoma. Molecular analyses were undertaken in seven cases of $CD10^{down}$ GI-FL, and an identical clone was found between $CD10^{down}$ follicles and $CD10^{+}BCL2^{+}$ neoplastic follicles. In the diagnosis of cases with $CD10^{down}$ $BCL2^{+}$ follicles, careful examination with molecular studies should be carried out.

Follicular lymphoma (FL) is the most common low-grade B-cell lymphoma followed by mucosa-associated lymphoid tissue (MALT) lymphoma.[1] Constitutive expression of the anti-apoptotic protein B-cell lymphoma 2 (BCL2) by t(14;18)(q32;q21)/*IGH-BCL2* is the hallmark of this tumor. In addition, it is an important immunohistochemical finding for the diagnosis of FL, in which BCL2 co-expresses with the germinal center (GC) marker CD10. However, in FL of the gastrointestinal (GI) tract, downexpression of CD10 is occasionally experienced, which results in a differential diagnostic problem with other low-grade lymphoma, although most of such cases usually accompany typical neoplastic follicles with the co-expression of CD10 and BCL2 ($CD10^{+}BCL2^{+}$) in the same lesion.

It was known that in nodal FL, downexpressed CD10 ($CD10^{down}$) is associated with high grade disease and aggressive clinical behavior.[2,3] In contrast, in the GI tract, most cases of $CD10^{down}$ FL were low-grade tumors.[4,5] The frequency and clinicopathological characteristics of $CD10^{down}$ FL in the GI tract have not been well studied.

Approximately 15% of FL occurs in extranodal sites,[6] and the GI tract is the most commonly affected site.[7,8] With the recognition of this FL variant and the development of endoscopic technology, the characteristics of GI-FL have been gradually revealed. Gastrointestinal FL is predominantly found in the second part of the duodenum, and certain GI-FL spread over the small intestine.[4,5,9] Although a case that showed a transformation from duodenal FL to diffuse large B-cell lymphoma (DLBCL) was reported,[10] GI-FL is generally an indolent disease, and certain cases showed spontaneous regression.[11] Duodenal FL shares some characteristics with MALT lymphoma, such as a lack of a follicular dendritic cell (FDC) meshwork, the deviation of VH usage, a lack of activation-induced cytidine deaminase, and differentiation to memory cells, which are different from nodal FL.[12] Furthermore, a genetic similarity between duodenal FL and MALT lymphoma was identified by comprehensive gene expression analysis.[13]

Considering these facts, downexpression of CD10 in GI-FL may have a different level of significance from that in nodal FL.

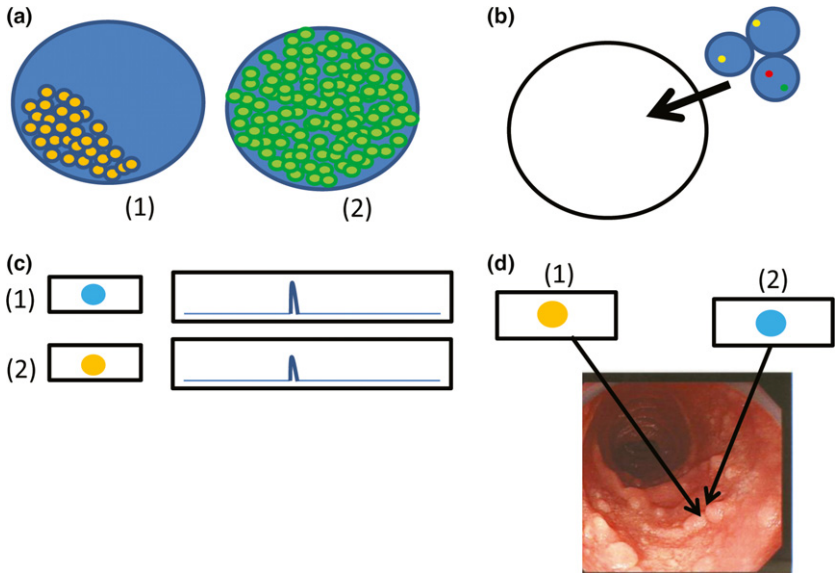

Fig. 1. Schema of criteria for CD10 downexpressed ($CD10^{down}$) follicular lymphoma. (a) Presence of the coexpression of CD10/BCL6 (1, orange dot) and BCL2 (2, green dot) in parts of the typical neoplastic follicles (blue circle) using immunohistochemistry. (b) Translocation of *IGH/BCL2* (yellow dot) in $CD10^{down}$ tumor follicles (white circle) using FISH. (c) Identical clone between $CD10^{down}$ follicles (1) and typical neoplastic follicles with the coexpression of CD10 and BCL2 (2) using PCR. (d) Presence of typical neoplastic follicles with coexpression of CD10 and BCL2 (1) and $CD10^{down}$ follicle (2) in different samples taken from the same lesion.

Table 1. Antibodies used in this study

Antibody	Source	Clone	Dilution
CD20	Novocastra, Newcastle Upon Tyne, UK	L26	1:200
CD3	Novocastra, Newcastle Upon Tyne, UK	LN10	1:50
CD10	Novocastra, Newcastle Upon Tyne, UK	56C6	1:50
BCL2	Novocastra, Newcastle Upon Tyne, UK	3.1	1:200
BCL6	Santa Cruz Biotechnology, Santa Cruz, CA, USA	D-8	1:100
CD21	Dako, Glostrup, Denmark	1F8	1:20
Cyclin D1	Nichirei, Tokyo, Japan	SP4	Ready to use
CAM5.2	Becton Dickinson, Franklin Lakes, IL, USA	CAM5.2	Ready to use

Therefore, in the present study, we investigated the clinical, morphologic, and immunophenotypical features of $CD10^{down}$ GI-FL and compared them with those of $CD10^{+}$ GI-FL.

Materials and Methods

Patient selection. The cases retrieved from the consultation files of the Department of Pathology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences (Okayama, Japan) included 172 cases of GI-FL (1993–2012), 144 cases of nodal FL (2012–2013), and 1024 cases of MALT lymphoma in the GI tract (1989–2013). Cases of FL *in situ*, grade 3 FL, and FL with a diffuse area or DLBCL in other sites were excluded from the present study.

$CD10^{down}$ FL was defined by one of the following findings (Fig. 1): (i) the presence of the co-expression of CD10/BCL6 and BCL2 in parts of the typical neoplastic follicles, which are composed of small- to medium-sized cells along with a few large-sized cells (grade 1–2); or (ii) the translocation of *IGH/BCL2* in $CD10^{down}$ tumor follicles using FISH; (iii) an identical clone verified between $CD10^{down}$ follicles and typical $CD10^{+}BCL2^{+}$ neoplastic follicles by PCR; or (iv) the presence of $CD10^{+}BCL2^{+}$ neoplastic follicles in different samples taken from the same lesion.

Complete clinical information was obtained from 58 of 172 cases of GI-FL. The International Workshop classification (Lugano classification)[14] was used for the clinical staging of GI-FL. Twenty-nine of 58 cases with stage I and II_1 were recognized as a primary GI-FL.[5] The site of involvement was based on biopsy or operation specimens. The Follicular Lymphoma International Prognostic Index was used for the evaluation of patient status.[15] The study protocol was approved by the Institutional Review Board of Okayama University (Okayama, Japan). All study procedures were

Table 2. Comparison of CD10 expression between nodal and gastrointestinal (GI) follicular lymphoma

	$CD10^{+}$	CD10 downexpressed	*P*-value
Nodal lesion (primary nodal) (n=144)	139	5	
GI lesion (n=172)	137	35	**<0.001**
Primary nodal (n=144)	139	5	
Primary GI (n=29)	13	16	**<0.001**

Bold values indicate significance. Downexpressed, ≤50% positive cells in neoplastic follicles; positive, >50% positive cells in neoplastic follicles.

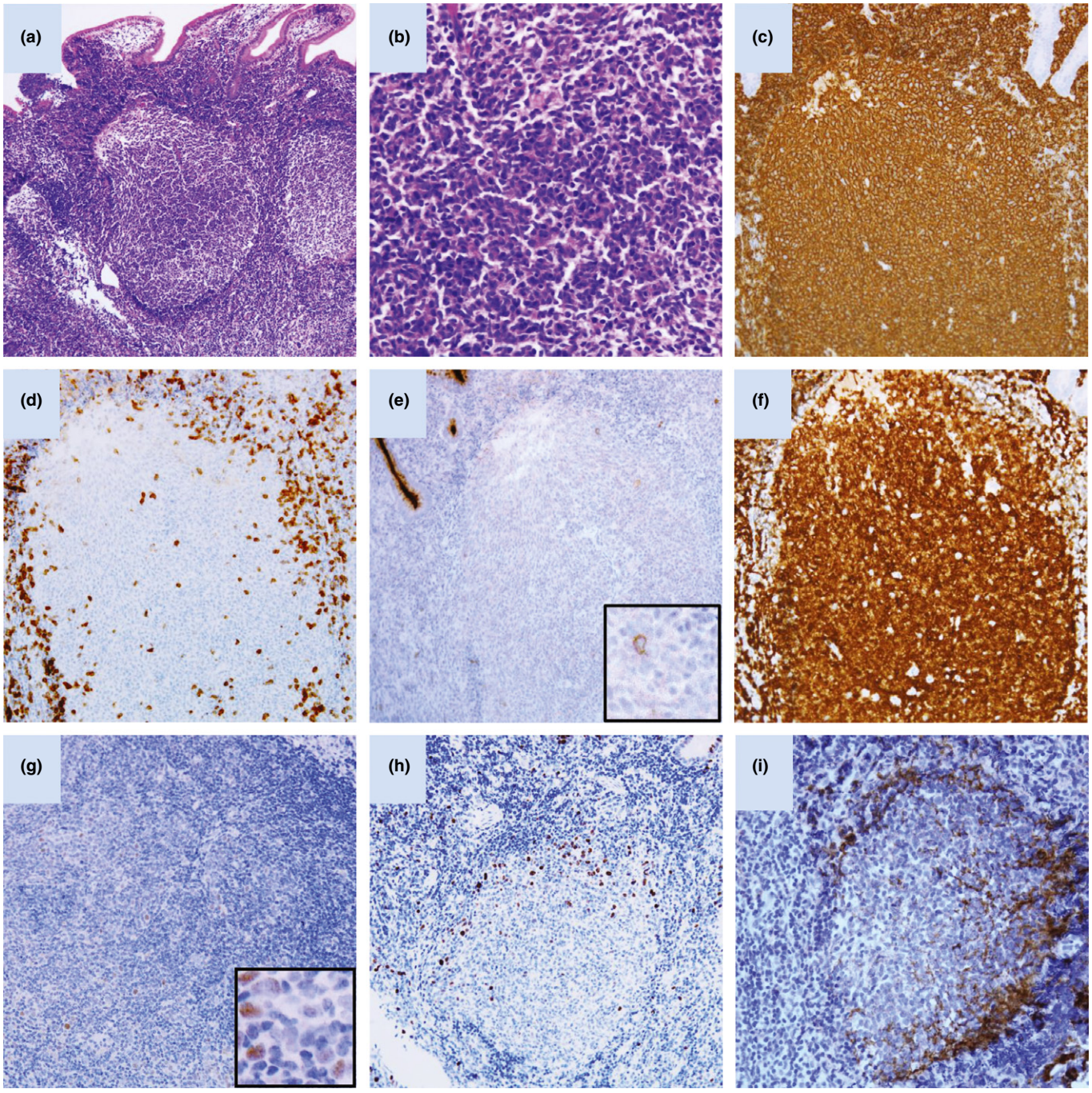

Fig. 2. Pathological features of CD10 downexpressed gastrointestinal follicular lymphoma (FL). (a) Neoplastic follicles are present in the lamina propria mucosa (HE, ×200). (b) Tumor cells were composed of a monotonous population of small- to medium-sized and a few large-sized cells (HE, ×400). (c) CD20 was positive, and (d) CD3 was negative in the neoplastic follicle. (e) CD10 was downexpressed in the neoplastic follicle (×400). (f) BCL2 was positive (×400). (g) BCL6 was downexpressed (×400). (h) A few Ki-67$^+$ cells were seen. (i) CD21$^+$ follicular dendritic cells present at the periphery of the neoplastic follicle (duodenal pattern).

carried out in accordance with guidelines of the Declaration of Helsinki.

Histology and immunohistochemistry. Histological and immunophenotypical features were studied on 10% formalin-fixed paraffin-embedded tissue sections (FFPET). Immunohistochemistry was carried out using an automated Bond-Max stainer (Leica Biosystems, Nussloch, Germany) according to the manufacturer's instructions. The primary antibodies used in the present study are summarized in Table 1. The expression of CD10 and BCL6 were scored as positive (>50% positive cells in neoplastic follicles) or downexpressed (≤50% positive cells in neoplastic follicles).

In the present study, samples that included no obvious follicles in sections were categorized as positive for CD10 and BCL6. The FDC pattern was classified as nodal (>30% positive FDC cells), intermediate (5–30% positive FDC cells), and duodenal (<5% positive FDC cells and FDC located at the periphery tumor follicles), as previously

Table 3. Comparison between CD10-positive and CD10 downexpressed FL

	CD10 positive *n*=137	CD10 downexpressed *n*=35	*P* value
Site of involvement of GI tract (all cases, *n*=172)			
Small intestine only	128	28	
Stomach or large intestine with/without small intestine	9	7	**0.015**
Site of involvement of GI (primary GI-FL cases, *n*=29)			
Small intestine only	13	13	
Stomach or large intestine with/without small intestine	0	3	0.099
Sex			
Male	16	15	
Female	15	12	0.76
Age: median(range)	59 (40–85)	63 (38–78)	0.75
Ann-Arbor staging			
1,2	23	18	
3,4	8	9	0.53
Lugano classification			
I–II1	13	16	
II2–IV	17	11	0.23
FLIPI			
Low	18	14	
Intermediate	7	7	
High	4	5	0.80
Anemia*			
Present	1	1	
Absent	29	24	0.90
LDH			
Normal	22	20	
Elevated	8	5	0.56
sIL-2R			
Normal	20	12	
Elevated	8	6	0.73
WBC (/μl)			
<9000	30	25	
9000<	0	1	0.28
Thrombocytopenia**			
Present	7	4	
Absent	23	21	0.50
Hypoalbuminemia***			
Present	2	2	
Absent	25	17	0.71
Hypocalcemia****			
Present	4	2	
Absent	14	21	0.22
Primary therapy			
Watchful wait	17	17	
R	3	2	
R-CHOP	4	2	
CHOP and CHOP-like	4	2	
operation or endoscopic resection	2	2	
Other	1	1	
Unknown		1	
Follow-up time: median (range) (month)	68 (7–157)	70 (1–201)	0.42
BCL6			
Positive	12	4	
Negative	5	22	**<0.001**
FDC pattern			
Duodenal	9	19	
Nodal	6	4	0.12

*Anemia; serum hemoglobin level <12 g/dL (male), <10 g/dL (female) **Thrombocytopenia, serum platelet level <150,000/μL ***Hypoalbuminemia, serum albumin level <3.9 g/dL ****Hypocalcemia, serum calcium level <8.6 mg/dL

described.[12] Three cases that showed both nodal and duodenal patterns were excluded from the comparison of FDC patterns, and three cases that showed both duodenal and intermediate patterns were classified as the duodenal pattern.

Polymerase chain reaction (*IGH*). DNA was extracted from FFPET, and PCR reactions were undertaken according to the BIOMED-2 Concerted Action protocol.[16] The *IGH* framework region 3 (FR3) primer was used as previously described.[12,16,17]

Fluorescence *in situ* hybridization for *IGH/BCL2*. To detect *IGH/BCL2* translocation for CD10 negative follicles, instant quality FISH was carried out using Agilent FISH General Purpose Reagents (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's instructions. Fluorescence *in situ* hybridization was carried out on FFPET, and the spectrum red-labeled *BCL2* probe (18q21.33 BCL2 DF 994 kb) and the spectrum green-labeled *IGH* probe (14q32.33 IGH DF 1519 kb P5) were used. Each case was interpreted as positive for *IGH/BCL2* translocation if a fusion signal was observed in more than 7% of nuclei, according to a previous report.[18]

Statistical analyses. Student's *t*-test or the χ^2-test was used. A *P*-value <0.05 was considered statistically significant. All statistical analyses were undertaken using the SPSS software package (version 14.0; SPSS, Chicago, IL, USA).

Results

Clinical features. The morphology and immunohistochemistry of $CD10^{down}$ GI-FL are shown in Fig. 2. The frequency and clinicopathological features of $CD10^{down}$ GI-FL and nodal FL are summarized in Tables 2 and 3.

A total of 172 cases of FL in the GI tract included 82 male and 90 female patients with a median age of 60.5 years (range, 37–85 years), and 144 cases of FL in the lymph node included 60 male and 84 female patients with a median age of 63.5 years (range, 32–84 years). Among each of the FL cases, 35 cases in the GI tract (20.3%, 18 men and 17 women) and five cases (3.5%, 5 women) in the lymph node were classified as within the $CD10^{down}$ group. CD10 downexpression was consequently significantly more frequent in GI-FL than in nodal FL (3.5%, $P < 0.001$; Table 2). In addition, when we confined the analysis to primary GI-FL, the difference was additionally significant between GI-FL and nodal FL (55.2% *vs* 3.5%, $P < 0.001$; Table 2).

The clinical information of GI-FL was obtained in 58 cases (31 $CD10^+$ GI-FL and 27 $CD10^{down}$ GI-FL). The median follow-up time of the $CD10^+$ GI-FL group was 68 months, and that of the $CD10^{down}$ GI-FL group was 70 months. More than 50% of cases were followed up without any treatment (watch and wait), and no patient died of the primary disease. $CD10^{down}$ GI-FL significantly involved the stomach and/or large intestine ($P = 0.015$), and all cases involved the small intestine. When confined to primary GI-FL, $CD10^{down}$ GI-FL tended to involve the stomach and/or the large intestine although it was not significant ($P = 0.099$; Table 3). There was no significant difference in other clinical characteristics.

In the present study, the entire gastrointestinal tract was examined using double balloon endoscopy and/or capsule endoscopy in 13 cases, and all involved the duodenum. Two cases (one $CD10^+$ case, one $CD10^{down}$ case) were localized in the duodenum, and 11 cases (five $CD10^+$ cases, six $CD10^{down}$ cases) involved the duodenum and other parts of the small intestine. There was no significant difference between these two groups in CD10 expression ($P = 0.91$) and other clinicopathological characteristics.

Pathological features. There was no difference in the morphology of $CD10^{down}$ GI-FL and $CD10^+$ GI-FL, and both showed neoplastic follicles composed of monotonous tumor cells of a small to medium size and a few large-sized cells without tingible body macrophages.

It was found that BCL6 was significantly downexpressed in $CD10^{down}$ GI-FL ($P < 0.001$). Duodenal pattern of FDC was often observed in $CD10^{down}$ GI-FL, although there was no significant difference ($P = 0.12$; Table 3). The pathological features of the 35 cases of $CD10^{down}$ GI-FL are shown in Table 4. The average proportion of $CD10^+$ and $BCL6^+$ tumor cells in the follicle were 12.6% (range, 0–50%) and 28.5% (range, 0–100%), respectively.

Lymphoepithelial lesions (LEL) in gastric lesions were observed in 5/12 (41.6%) cases (Fig. 3). The macroscopic

Table 4. Immunohistochemical and immunogenotypical results in 35 cases of CD10 downexpressed follicular lymphoma

Patient no.	Age, years	Sex	Site of involvement†	Proportion of $CD10^+$ cells,‡ %	Proportion of $BCL6^+$ cells,‡ %	PCR*	FISH**	$CD10^+$ lesion***	CD21
1	73	M	*SI*	0	ND	1	ND	1	Duodenal
2	73	M	*SI*	10	100	1	ND	1	Nodal
3	67	M	*SI*	20	20	1	ND	2	Mismatch
4	66	F	*SI*	50	<10	1	ND	1	ND
5	54	M	St, *SI*, LI	20	10	1	ND	1	Duodenal
6	61	M	*SI*	0	0	1	ND	1	Duodenal
7	51	M	*SI*	0	20	1	ND	1	Duodenal
8	54	M	St, *SI*	<10	30	2	+	1	Mismatch
9	66	F	*SI*	20	20	2	+	1	ND
10	37	F	*SI*	<10	0	ND	+	1	ND
11	55	M	*SI*	0	100	2	ND	1	Duodenal
12	69	F	*SI*	50	<10	2	ND	1	Duodenal
13	63	M	*SI*	0	0	2	+	2	Duodenal
14	52	M	*SI*	0	100	2	ND	1	Duodenal
15	54	M	*SI*	0	15	2	ND	1	Nodal
16	69	F	*SI*	30	0	ND	ND	1	Duodenal
17	61	F	*SI*	0	100	ND	ND	1	Duodenal
18	73	F	*SI*	40	10	ND	ND	0	Duodenal
19	56	M	*SI*	0	30	ND	ND	1	ND
20	76	F	*SI*	30	30	ND	ND	1	Mismatch
21	68	M	*SI*	20	ND	ND	ND	1	ND
22	55	F	*SI*	30	ND	ND	ND	1	Duodenal
23	75	F	*SI*	20	ND	ND	ND	1	ND
24	48	M	St, *SI*	0	15	ND	ND	1	ND
25	65	M	*SI*	0	ND	ND	ND	1	Nodal
26	57	F	*SI*	10	ND	ND	ND	1	ND
27	64	F	*SI*	0	20	ND	ND	1	Duodenal
28	78	F	*SI*	30	ND	ND	ND	1	Duodenal
29	58	M	*St*, SI	0	20	ND	ND	1	Nodal
30	38	F	St, *SI*, LI	0	30	ND	+	1	Duodenal
31	46	F	*SI*	0	10	ND	ND	1	Duodenal
32	45	M	St, SI, *LI*	20	ND	ND	ND	1	ND
33	70	F	*SI*	20	0	ND	ND	1	Duodenal
34	62	M	*St*, SI	0	50	ND	ND	2	Duodenal
35	49	F	*SI*	10	ND	ND	ND	1	Duodenal

LI, large intestine; ND, not done; SI, small intestine; St, stomach. †Italic text indicates sites where CD10 downexpressed follicles were seen. ‡<10 indicates <10% $CD10^+$ or $BCL6^+$ tumor cells. *1, identical clone between CD10 downexpressed follicles and typical CD10 and BCL2 positive neoplastic follicles; 2, negative in both CD10 downexpressed follicles and typical CD10 and BCL2 positive neoplastic follicles. **+, positive for a IgH/BCL2 translocation (a fusion signal was observed in more than 7% of nuclei). ***0, the case without CD10 positive neoplastic follicles in the gastrointestinal (GI) tract; 1, the case with CD10 positive neoplastic follicles in same lesion; 2, the case with CD10 positive neoplastic follicles in another GI lesion.

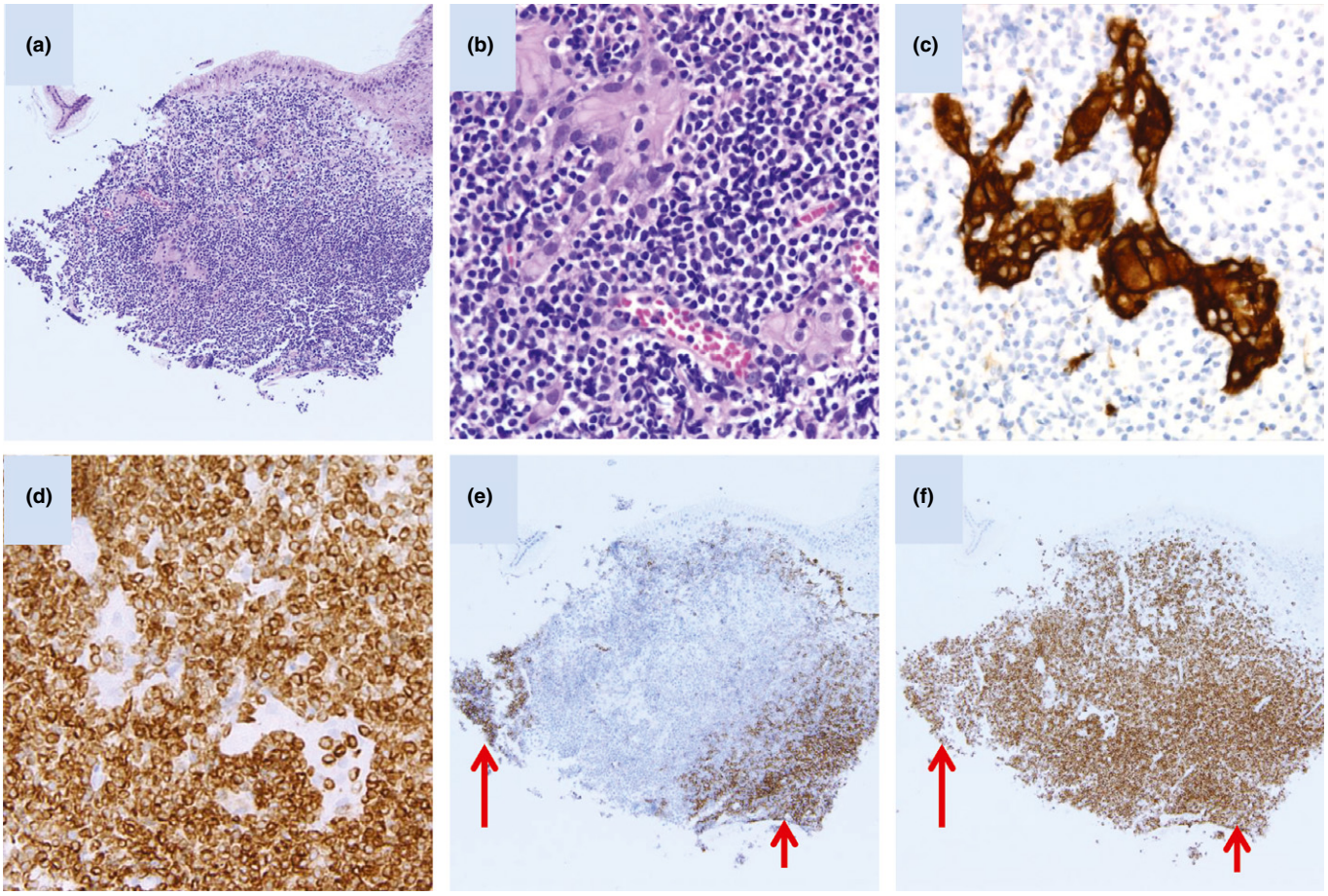

Fig. 3. Representative case of gastric follicular lymphoma with lymphoepithelial lesion (LEL). (a) Diffuse proliferation of small- to medium-sized cells were seen in the proper mucosa (HE, ×100). (b–d) LEL with neoplastic B cells. (b) HE, ×400. (c) CAM 5.2 staining, ×400. (d) CD20 staining, ×400. (e) CD10 was positive (arrows) but downexpressed around the LEL area. (f) BCL2 was strongly positive in both $CD10^{+}$ (arrows) and CD10 downexpressed areas.

findings of these five cases were as follows: two cases presented with a submucosal tumor-like lesion, one with multiple nodules, one with multiple nodules on a flat elevated lesion, and one was unknown. Three cases were in the $CD10^{+}$ group, and the remaining two cases were in the $CD10^{down}$ group.

Molecular features. The results of PCR and FISH in 35 cases of $CD10^{down}$ GI-FL are shown in Table 4. Polymerase chain reaction analysis was undertaken for 14 cases. Seven cases were successfully amplified, and in all cases, an identical clone was found between $CD10^{down}$ follicles and $CD10^{+}BCL2^{+}$ neoplastic follicles (Fig. 4a,b). Five cases were examined for further FISH analysis (three duodenal lesions and two ileal lesions), and all five cases of $CD10^{down}$ GI-FL showed *IGH/BCL2* translocation (Fig. 4c). The diagnostic algorithm in $CD10^{down}$ GI-FL is shown in Fig. 5.

Discussion

In the present study, we found that CD10 downexpression was frequently observed along with an additional distinct feature of GI-FL. $CD10^{down}$ GI-FL frequently involved the stomach or large intestine, and showed the simultaneous downexpression of BCL6. Furthermore, gastric FL occasionally contained LEL in parts of the lesions.

For the diagnosis of $CD10^{down}$ FL, a careful differentiation of the follicular colonization of MALT lymphoma and normal primary follicles is always required because both have identical immunohistochemical features ($CD10^{-}/BCL6^{-}$ and $BCL2^{+}$). Follicular colonization is the phenomenon in which MALT lymphoma invades to reactive GC and closely resembles FL.[8,19,20] In the present study, we carefully excluded MALT lymphoma showing this phenomenon, and diagnosed $CD10^{down}$ FL by one of the following findings: (i) the presence of the co-expression of CD10/BCL6 and BCL2 in parts of the typical neoplastic follicles that are composed of small- to medium-sized and a few large-sized cells (grade 1–2); (ii) the translocation of *IGH/BCL2* in $CD10^{down}$ tumor follicles by FISH; (iii) an identical clone between $CD10^{down}$ follicles and $CD10^{+}BCL2^{+}$ neoplastic follicles using PCR; or (iv) the presence of $CD10^{+}BCL2^{+}$ neoplastic follicles in different samples taken from the same lesion. In the diagnosis of these cases, as shown in the algorithm for the diagnosis of $CD10^{down}$ GI-FL, it is important to carefully search for the evidence of FL. Following the above criteria, the addition of BCL6 staining, the re-biopsy of several areas, and the examination of the translocation of *IGH/BCL2* using FISH, and an identical clone verified between $CD10^{down}$ follicles and typical $CD10^{+}BCL2^{+}$ neoplastic follicles using PCR is required.

Similarity between GI-FL and MALT lymphoma was described in several previous studies. Both were frequently present within localized diseases, resulted in indolent clinical behavior,[21–23] and shared gene expression profiles.[12,13]

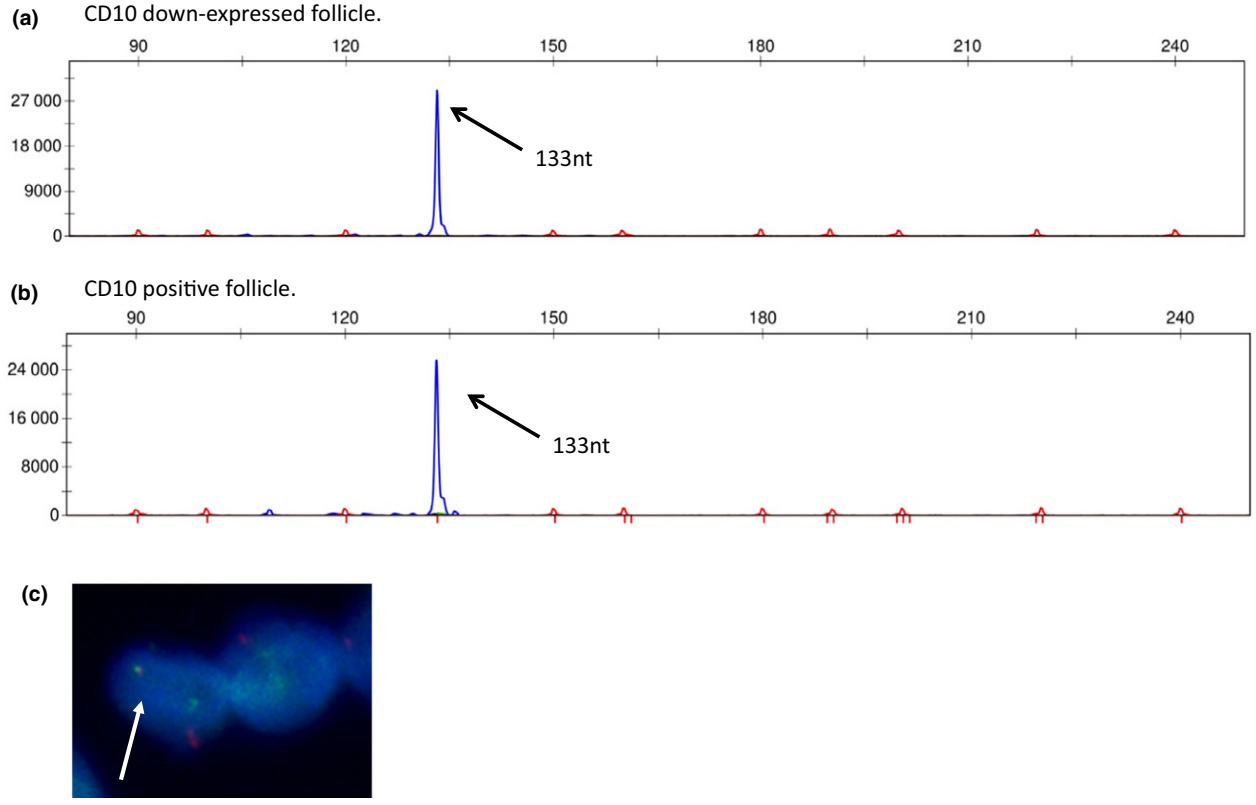

Fig. 4. (a,b) Polymerase chain reaction for immunoglobulin heavy chain rearrangements in with gastrointestinal follicular lymphoma. Identical clone (133 nt) was detected between CD10 downexpressed (a) and $CD10^{+}BCL2^{+}$ follicles (b) in the same patient. (c) FISH for *IGH/BCL2* translocation in CD10 downexpressed tumor follicle. Fusion signal for *IGH/BCL2* (yellow signal; arrow) was seen.

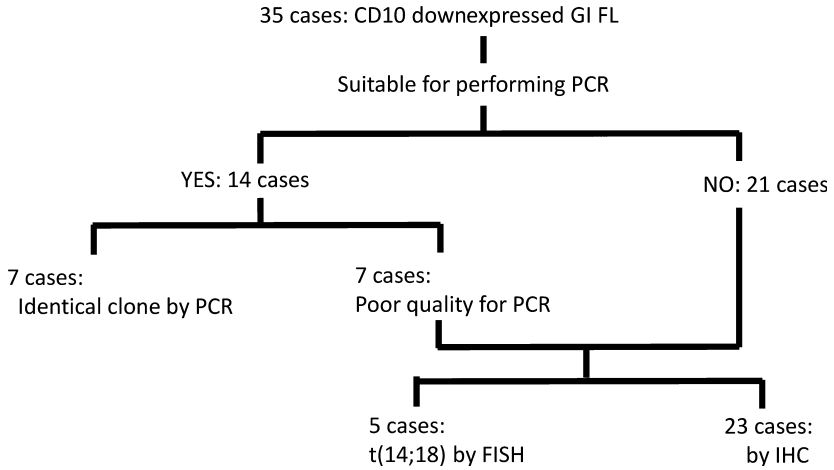

Fig. 5. Algorithm of diagnosis in CD10 downexpressed gastrointestinal follicular lymphoma (GI-FL). IHC, immunohistochemistry.

Interestingly, it has been reported that, similar to MALT lymphoma, certain GI-FL showed partial regression after antibiotic treatment.[9,24,25] The frequent downexpression of GC markers (CD10 and BCL6) in GI-FL of the present study was consistent with these previous data.

In the present study, LEL was shown in 40% of FL with gastric lesion. It is considered that LEL is a characteristic finding of MALT lymphoma in which lymphoma cells have invaded and destroyed the epithelium.[8] It was of interest that a few cases of GI-FL additionally showed LEL. It should be kept in mind that LEL itself was not a MALT-specific phenomenon.

Follicular lymphoma with marginal zone differentiation is known to show morphological features, such as marginal zone lymphoma (MZL) in parts of FL, and have both $CD10^{+}BCL2^{+}$ neoplastic follicles of FL and the morphology of MZL, with monocytoid or plasmacytoid B cells in the interfollicular area.

Table 5. Tumor distribution among cases of CD10$^+$ follicular lymphoma (FL), CD10 downexpressed FL, and MALT lymphoma

	Esophagus	Stomach	Small intestine	Large intestine
FL				
CD10$^+$ (n = 137)	0	5	133	4
CD10 downexpressed (n = 35)	0	7	35	3
MALT lymphoma (n = 1024)	2	815	32	176

It is considered that this is not composite lymphoma, but that the two components are clonally related.[26,27] Follicular lymphoma with LEL could be considered as FL with marginal zone differentiation because parts of them have a similar morphology to MZL. These cases might be misdiagnosed as MALT lymphoma if a CD10$^+$ area is not obtained in the small biopsy specimen. Therefore, FL with marginal zone differentiation is one of the important differential diagnoses of MALT lymphoma, particularly in patients with a history of FL.

Macroscopic findings of typical FL in the small intestine were described as multiple white nodules,[4,9] and FL in the stomach could show various appearances. In MALT lymphoma in the stomach, various appearances were observed such as flat, elevating, and ulcerative lesions, however, multiple nodules were extremely rare.[23,28,29] In the present study, two of four cases of FL with LEL did not show characteristic macroscopic appearances of MALT lymphoma. Considering these facts, when we diagnose MALT lymphoma with LEL showing an endoscopically atypical appearance of MALT lymphoma, careful examination should be required to avoid misdiagnosis.

We have previously reported a case that was initially diagnosed as MALT lymphoma in the ileum and FL in the jejunum but was subsequently diagnosed as FL with marginal differentiation.[30] Although only a diffuse proliferation of medium-sized CD10$^-$BCL2$^+$ cells was observed in the ileal lesion, it was shown that both lesions had *IGH*/*BCL2* using FISH and an identical band was found using Southern blot. In the present study we categorized the CD10^{down} group as those with obvious follicles with ≤50% CD10$^+$ cells. However, there would be FL cases with CD10$^-$ cells with only diffuse proliferation like this case report. Considering these facts, the number of CD10^{down} FL cases could be more than what we analyzed.

Table 5 shows the site of involvement of CD10^{down} GI-FL, CD10$^+$ GI-FL, and MALT lymphoma. Among the 1024 MALT lymphoma cases, 815 cases (79.6%) involved the stomach, 176 cases (17.2%) involved the large intestine, and 32 cases (3.1%) involved the small intestine. In contrast to MALT lymphoma, the majority of CD10$^+$ FL cases (97.1%) involved the small intestine, and the involvement of the stomach or large intestine was extremely rare. In CD10^{down} GI-FL, although all cases were presented with small intestinal lesion, they additionally tended to involve the stomach (20%) or large intestine (5.7%). As described above, this phenomenon may be one of the characteristics in CD10^{down} GI-FL. Because most of the cases were diagnosed by biopsy in the present study, the distribution (diffuse or focal) of CD10^{down} neoplastic follicles is difficult to describe, as this would require an examination of the distribution of CD10^{down} neoplastic follicles by several biopsies over a wide area in the same organ using immunohistochemistry and molecular study. Therefore, this aspect is a subject for future research.

Further discussion is required regarding the cut-off value of CD10 in GI-FL. In one previous report, the cut-off value of CD10 was 30%[12]; however, the cut-off values of CD10 in several reports were not explicitly set.[4,31] Furthermore, in a previous report regarding CD10 expression in primary intestinal DLBCL, CD10 staining was scored simply as positive or negative without setting the cut-off value.[32] It is suggested that there is currently no consensus on the cut-off value of CD10 in GI lymphomas. We reclassified FL by setting the cut-off value of CD10 downexpressed at 30% and repeated the comparison between the clinicopathological characteristics of CD10$^+$ and CD10^{down} FL. As a result, a similar tendency was observed: CD10 downexpression was significantly more frequent in GI-FL than in nodal FL ($P < 0.001$). Even if confined to primary GI-FL, the difference was also significant between GI-FL and nodal FL ($P < 0.001$). CD10^{down} GI-FL significantly involved the stomach and/or large intestine ($P = 0.007$), and BCL6 was significantly downexpressed in CD10^{down} GI-FL ($P = 0.004$). We believe that this occurred because the proportion of CD10$^+$ cell was <30% in most CD10^{down} cases, as shown in Table 4. In addition, unlike biopsy specimens of lymph nodes, biopsy specimens of GI lymphomas are very small and easily crushed. Based on these facts, we set the cut-off value of CD10 downexpressed to 50% to prevent a complicated diagnosis and to improve reproducibility.

In conclusion, CD10 downexpression was more frequent in GI-FL than in nodal FL and significantly involved the stomach or large intestine. Furthermore, gastric FL occasionally had LEL in part of the lesion. In the diagnosis of cases with CD10^{down} BCL2$^+$ follicles, molecular analysis should be undertaken. Further prospective studies are warranted to clarify the pathophysiology of GI-FL.

Disclosure Statement

The authors have no conflict of interest.

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