Original Article

A20 (TNFAIP3) Alterations in Primary Intestinal Diffuse Large B-cell Lymphoma

Masayoshi Fujii[a,b], Katsuyoshi Takata[a]*, Shih-Sung Chuang[c]*, Tomoko Miyata-Takata[a], Midori Ando[d], Yasuharu Sato[a], and Tadashi Yoshino[a]

[a]Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan, [b]Department of Pathology, St. Mary's Hospital, Himeji, Hyogo 670-0801, Japan, [c]Department of Pathology, Chi-Mei Foundation Hospital, Tainan 710, Taiwan, [d]Department of Pathology, Kagawa Prefectural Central Hospital, Takamatsu 760-8557, Japan

The gastrointestinal (GI) tract is the most frequently involved site of extranodal non-Hodgkin lymphomas, and diffuse large B-cell lymphoma (DLBCL) is the most common subtype occurring in the GI tract. *TNFAIP3 (A20)* genetic alterations were reported to be involved in DLBCL's pathogenesis and a portion of GI-DLBCL cases harbor this alteration. However, the frequency and clinicopathological relations focusing on small and large intestinal DLBCL are unclear. Here, we examined *A20* deletion and protein expression and analyzed the clinicopathological features of 52 cases of primary intestinal DLBCL. The most frequently involved site was the ileocecal region (75%), followed by small bowel (13.5%) and large intestine. Immunohistochemically, the ileocecal cases expressed BCL6 ($p = 0.027$) and MUM1 ($p = 0.0001$) significantly more frequently than the small intestinal cases. Six of 47 cases (13%) had *A20* heterozygous deletion, whereas all 6 heterozygously deleted cases had detectable A20 protein expression. In summary, *A20* abnormality was less prevalent among intestinal DLBCLs with some discordancy between gene deletion and protein expression. Although the *A20* alteration status did not affect any clinicopathological characteristics in this series, further studies exploring alterations of *A20* and other NF-κB components in primary intestinal DLBCL are needed.

Key words: primary intestinal diffuse large B-cell lymphoma, cell of origin, *A20*, *TNFAIP3*, heterozygous deletion

T
he gastrointestinal (GI) tract is the most commonly involved site of extranodal non-Hodgkin's lymphomas. The predominant subtypes of lymphomas are different according to the site of the GI tract: mucosa-associated lymphoid tissue (MALT) lymphoma for the stomach (46.2%) [1], follicular lymphoma for the duodenum (38%) [2], and diffuse large B-cell lymphoma (DLBCL) for the small and large intestines (41%) [3]. DLBCL of the small intestine is a rare disease but sometimes causes abdominal pain, intestinal obstruction or perforation. Our studies focusing on clinicopathological features of primary intestinal DLBCL revealed that perforation was one of the independent poor prognostic factors in addition to the predominance of activated B-cell (ABC) phenotype [4,5].

A20, also known as tumor necrosis factor alpha-induced protein 3 (*TNFAIP3*), is located on chromosome band 6q23 and negatively regulates the NF-κB pathway, which is dysregulated by several types of genetic alterations including oncogenic mutations of *MALT1* and *CARD11* [6]. Deletion of *A20* (*TNFAIP3*) was reported

in several types of lymphoma such as DLBCL of the ABC phenotype, MALT lymphoma, Hodgkin lymphoma, and Epstein-Barr virus (EBV)-associated lymphoma. A20 is a dual-function enzyme that adds and subtracts ubiquitin moieties to deactivate and degrade receptor-interacting protein (RIP), an essential mediator of the proximal TNFR1 signaling complex [7]. A20 restricts toll-like receptor-induced NF-κB signals by deubiquitylation and also regulates MAP kinase signaling cascades. Further, A20 restrains inflammatory responses and acts as an anti-apoptotic factor, regulating influences on immune homeostasis [8].

Although approx. 20% of DLBCL cases were reported to carry abnormalities in the *A20* gene [9], the frequency of *A20* alterations and its clinicopathological significance in intestinal DLBCL are not characterized. In the present study, we investigated *A20* deletion and protein expression and analyzed their significance in 56 cases of primary intestinal DLBCL.

Materials and Methods

Patient selection. Fifty-eight surgically resected intestinal DLBCL samples (obtained from 1990 to 2012, Chi-Mei Foundation Hospital, Taiwan and affiliated hospitals) were used in this study, and they are the same cases series used in the report by Lu *et al.* [5]. We applied strict inclusion criteria for primary intestinal lymphoma according to the American Joint Committee on Cancer Staging Manual, which is a modification of the Ann Arbor system. We excluded cases of secondary involvement from other organs in this study. The intestinal site was classified according to the criteria of Koch *et al.*: duodenum, small intestine, ileocecum, colon, and rectum [10]. The histologic diagnosis of each case was made according to the criteria by the current World Health Organization classification [11].

A total of 56 formalin-fixed paraffin embedded tissue (FFPET) samples were used for tissue microarray, and two whole resected FFPETs were used for immunohistochemical (IHC) and genotypic studies. Our study was approved by the Ethics Committee and Institutional Review Board of Okayama University Hospital (ID no. 493) and Chi-Mei Medical Centre (ID no. 10102-016).

FISH analysis for A20. *A20* deletion was investigated by dual-color fluorescence *in situ* hybridization (FISH) on FFPETs using s spectrum orange-labeled A20 probe (BAC clone RP11-783B20) and spectrum green-labeled centromeric probe for chromosome 6 (CEP6) (Vysis/Abbott Molecular Laboratories, Des Plaines, IL, USA) according to the manufacturers' instructions [12]. The cells were scored only when 2 internal positive control signals (CEP6) were present, and the signal ratio of *A20* and *CEP6* was calculated to evaluate the *A20* status. In DLBCL, the threshold for determining *A20* homozygous deletions was the fraction of signals ranging from 20% to 60%, and that for heterozygous deletions was from 60% to 80% as described [13].

IHC analysis. IHC staining was performed using an automated Bond Max autostainer (Leica Biosystems, Melbourne, Australia) and a Ventana XT autostainer (Ventana Medical Systems, Tucson, AZ, USA). The clones and dilutions of the primary antibodies used for this study were as follows: A20 (EPR2663, [1 : 100], Epitomics, Burlingame, CA, USA), CD20 (L26, [1 : 200], Novocastra Laboratories, Newcastle Upon Tyne, UK), CD10 (56C6, [1 : 50], Novocastra), BCL6 (D8, [1 : 250], Santa Cruz, CA, USA), MUM1 (MUM1p, [1 : 50], Dako, Glostrup, Denmark), BCL2 (Bcl-2, [1 : 40], Dako), c-MYC (9E10, [1 : 50], Santa Cruz).

In accord with previous reports, tumors that were comprised of at least 20% of A20-positive cells were scored as positive [14]. When the internal positive control cells were not clearly positive for A20, the sample was classified as "indeterminate" or "equivocal." In the "undetermined" groups, the tumor cells were negative, and in the "equivocal" groups, the tumor cells were weakly positive.

Statistical analysis. Overall survival (OS) was measured from the date of diagnosis to death from any cause. Event-free survival (EFS) was not available because of paucity of data of most cases. Survival curves were generated by the Kaplan-Meier method, and the value was compared by log-rank test. Fisher's exact test or chi-squared test was used. We considered p-values < 0.05 significant.

Results

Patient characteristics. Clinical data was available for 52 of the 58 patients, including 29 men (56%) and 23 women (44%). The median age was 64 years (range, 23-87 years). The tumor sizes ranged from 2.4 to 16 cm with a mean size of 8.4 cm. The most fre-

Table 1 Clinicopathological features of 52 Primary Intestinal DLBCL cases

Factors		No. of cases		Factors			No. of cases
Age				Immunohistochemical status			
	< 59	18		A20	−		4
	≧ 60	34			+		48
Sex				CD10	−		40
	man	29			+		12
	woman	23		BCL2	−		14
Sites of origin					+		38
	duodenum	2		BCL6	−		9
	small intestine	7			+		43
	ileocecum	39		MUM1	−		12
	Colon	1			+		39
	rectum	1		c-MYC	−		37
	multiple intestinal sites	2			+		13
Tumor morphology				Subtypes by Hans classification			
	centroblastic	49				GCB	16
	Immunoblastic	3				non-GCB	36
	other	0		A20 status by FISH			
Tumor size						no deletion	41
	< 8 cm	27				heterozygous deletion	6
	≧ 8 cm	19		Lugano stage			
Depth						I	22
	< subserosa	21				II	30
	≧ serosa	24		PS			
Perforation						0~1	24
	no	42				2~4	4
	yes	10		IPI			
Ulcer						0~1	13
	no	37				2~5	12
	yes	5		Adjuvant chemotherapy			
Serum LDH elevation						no	18
	no	23				yes	34
	elevated	11					

LDH, lactate dehydrogenase; PS, performance status; IPI, International Prognostic Index.

quently involved site was the ileocecal region (39 of 52 patients, 75%), followed by the small bowel (jejunum and ileum, 7 of 52 patients, 13.5%) (Table 1). Two patients had multiple sites of tumors: one involving the small intestine and colon, and the other involving the duodenum, small intestine and ileocecal region. The median follow-up time was 21 months (range, 0.2-210 months).

Pathological features. According to the DLBCL tumor morphology, IHC findings were evaluable for 52 samples, and the cell-of-origin was determined using Hans' algorithm. Sixteen cases (31%) were GCB-type, and 36 (69%) were non-GCB-type (Figs. 1 and 2). Ileocecal samples were significantly more frequently positive for BCL6 ($p = 0.027$) and MUM1 ($p = 0.0001$)
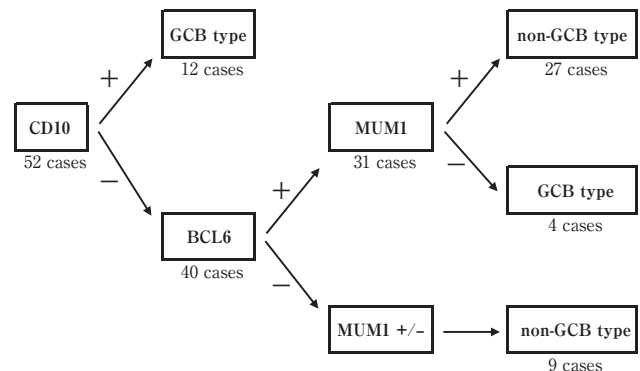

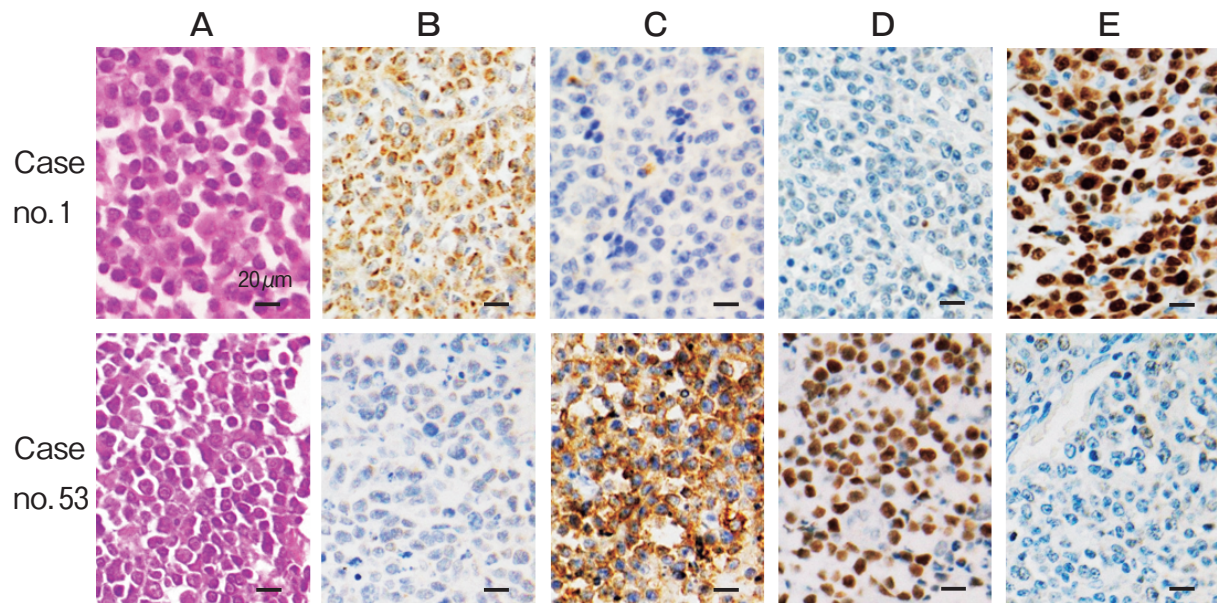**Fig. 1** Immunohistochemical subtypes according to Hans criteria.

Fig. 2   Tissue array samples (2 cases) of hematoxylin-eosin stain (**A**) and immunostains (**B**, A20; **C**, CD10; **D**, BCL6; **E**, MUM1), (Olympus BX51).
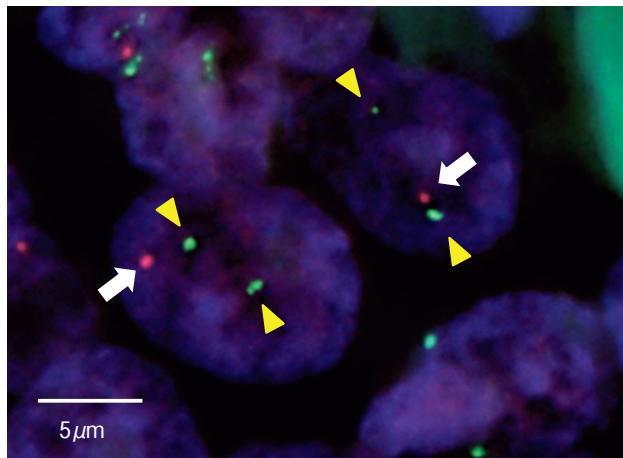

Fig. 3   FISH analysis of DLBCL case performed with a combination of *A20* probe (orange) and chromosome 6 centromeric probe (green). Two green signals (yellow arrowheads) and one red signal (white arrows) are seen in one cell, indicating heterozygous deletion of the *A20* gene (Olympus IX71).

compared to the small intestinal cases. Thirty-eight of the 52 (73%) samples were positive for BCL2, and 13 of 15 (26%) were positive for MYC. Six (12%) cases were BCL2 and MYC double-expressing DLBCL cases.

A20 deletion.   Forty-seven of the 52 samples showed evaluable FISH signals. Six of 47 cases (13%) had *A20* heterozygous deletion (Fig. 3), whereas there

was no case with homozygous deletion. The clinicopathological features of these six *A20*-deleted cases are summarized in Table 2. The median age of the 6 patients (4 men, 2 women) was 63 (range 51-81 years). The most frequently involved site was the ileocecal region (four cases, 67%) with a mean tumor size of 8.2 cm. Perforation was detected in 2 cases (33%), which led to death in both cases within 7 months. In a median follow-up time of 9 months, 4 patients (67%) were dead. In a univariate analysis, there were no significant differences between *A20*-heterozygously deleted and non-deleted cases including OS ($p = 0.63$). The clinicopathological features of these 6 heterozygous *A20* deletion cases and the 41 deletion-negative cases are summarized in Table 3.

IHC findings and their correlation with clinico-pathological characters and prognosis.   Forty-six of 52 samples were evaluable for the A20 IHC analysis. Forty-two samples (91%) were positive, and the remaining 4 samples (9%) were negative. All 6 cases with a heterozygous *A20* deletion were positive for A20 IHC.

Of the six *A20*-heterozygously-deletion cases, 5 were non-GCB-type and one was GCB-type. The median age of the 4 patients (2 men, 2 women) with negative A20 IHC was 63 (range 61-83 years). The ileocecal region (3 cases, 75%) was the most involved site, with a mean tumor size of 7.0 cm. Of these 4 patients, there was no

Table 2   Clinicopathological features of *A20* heterozygous deleted cases

Case no.	Age	Sex	Tumor site	Tumor size (cm)	Perforation	Immunohistochemical status						Hans classification	Serum LDH elevation	Adjuvant chemotherapy	Survival status	Followed month
						A20	CD10	BCL2	BCL6	MUM1	c-MYC					
1	51	woman	Small bowel (jejunum)	6.0	none	+	−	−	−	+	−	non-GCB	no	R-CHOP	alive	11
20	65	woman	Ileocecal region	5.0	none	+	−	+	+	+	−	non-GCB	no	CHOP	alive	135
28	81	man	Ileocecal region	5.5	none	+	−	+	+	+	−	non-GCB	no	R-CHOP	dead	23
32	60	man	Multiple (jejunum and colon)	6.0	yes	+	−	+	+	−	−	GCB	ND	R-CHO	dead	7
37	59	man	Ileocecal region	10.5	none	+	−	+	+	+	−	non-GCB	elevated	none	dead	6
39	78	man	Ileocecal region	16.0	yes	+	−	+	+	+	+	non-GCB	ND	none	dead	7

LDH, lactate dehydrogenase; ND, Not Done; CHOP, cyclophosphamide, doxorubicin, vincristine and predonisone; R-CHOP, Rituximab and CHOP.

intestinal perforation or mortality in a median follow-up time of 57 months. Of these A20 IHC-negative patients, 2 were non-GCB-type and 2 were GCB-type.

The adjuvant chemotherapies were performed with the regimens of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) (12 cases, 39%), CHOP (4 cases, 13%), CEOP (etoposide substituted for doxorubicin) (8 cases, 26%), and COP (4 cases, 13%). Other regimens included R-hyper CVAD (rituximab with hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone, 1 case, 3%), rituximab, etoposide and cyclophosphamide (1 case, 3%), rituximab and cyclophosphamide (1 case, 3%). The clinicopathological factors were not significantly different among the six *A20*-deleted, four A20 IHC-negative, and the remaining 41 patients, probably because the sample sizes of the former 2 groups were too small.

Discussion

We examined *A20* deletion and protein expression using primary intestinal DLBCL cases. *CARD11* and *MYD88* mutations are involved in the NF-κB pathway alterations including *A20*, and these are frequent events in non-GCB type DLBCL. We found that 6 of 52 cases (13%) showed *A20* heterozygous deletion, but all these cases were positive for A20 protein expression. On the other hand, 4 of the 52 cases were negative for A20 protein expression, whereas no *A20* deletion was observed. We demonstrated that *A20* deletion did not necessarily cause A20 protein down-expression [13]; some cases with heterozygous deletion showed A20 IHC positivity although cases with homozygous deletion showed A20 IHC negativity. It has also been reported that not only *A20* deletion but also *A20* promoter hypermethylation could contribute to *A20* down-expression [15]. Therefore, in the 4 present *A20* non-deleted and IHC-negative cases, the IHC negativity might have been caused by promoter hypermethylation.

We hypothesized that only *A20* haplo-insufficiency from heterozygous deletion might cause lymphomagenesis in B-cell lymphoma. Honma *et al.* reported that monoallelic *A20* inactivation as well as biallelic *A20* inactivation was frequently found in ABC-phenotype DLBCLs and mantle cell lymphomas, and they demonstrated that *A20* induced resistance to apoptosis and increased colony formation ability in human EB-LCL

Table 3   Clinicopathological features of *A20* heterozygous deleted cases vs. deletion negative cases*

		A20 status by FISH		
		heterozygous deletion	no deletion	*P*-value**
Total		6	41	
Age				
	<59	2	13	1
	≧60	4	28	
Sex				
	man	4	25	1
	woman	2	16	
Tumor site				
	Duodenum	0	2	
	Small intestine	3	11	
	Ileocecum	2	24	0.322
	Colon	0	2	
	Rectum	0	1	
	Mutiple sites	1	1	
Tumor size				
	<8 cm	4	20	0.685
	≧8 cm	2	16	
Perforation				
	none	4	35	0.267
	yes	2	6	
Immunohistochemical status				
A20	−	0	4	1
	+	6	37	
CD10	−	6	31	0.317
	+	0	10	
BCL2	−	1	12	1
	+	5	29	
BCL6	−	1	7	1
	+	5	34	
MUM1	−	1	9	1
	+	5	31	
c-MYC	−	5	32	1
	+	1	7	
Subtypes by Hans classification				
	non-GCB type	5	28	0.653
	GCB type	1	13	
serum LDH elevation				
	no	3	18	1
	yes	1	8	
adjuvant chemotherapy				
	None	2	14	
	R-CHOP	3	9	
	CHOP	1	3	1
	CEOP	0	8	
	COP	0	4	
	Others	0	3	
Survival status				
	alive	2	23	0.398
	dead	4	18	
Followed months				
	<20 months	4	16	0.379
	≧20 months	2	25	

LDH, lactate dehydrogenase; CHOP, cyclophosphamide, doxorubicin, vincristine and predonisone; R-CHOP, Rituximab and CHOP; CEOP, cyclophosphamide, etoposide, vincristine and predonisone; COP, cyclophosphamide, vincristine and predonisone.
*Insufficient 5 cases by FISH analysis were excluded.
**Fisher's Exact test.

(Epstein Barr virus lymphoblastoid cell line) even with a partial knockdown [15]. These results might support the role of *A20* gene in our cohort.

We also speculated that not only *A20* heterozygous deletion but also other gain-of-function mutations such as *CARD11* and *MYD88* which are frequently found in ABC-type DLBCLs could affect the lymphomagenesis. Although the cell types were different, Wolfrum *et al.* reported that pro-atherosclerotic NF-κB target gene was elevated and atherosclerosis was increased in *A20* haplo-insufficient mice, so that *A20* resulted in NF-κB deregulation [16]. This result might support the relationship between *A20* and NF-κB.

Because we had only paraffin-embedded samples for tissue microarray, our study was strictly limited to immunohistochemical and FISH analyses. We could not obtain further data including those from a real-time reverse transcription-polymerase chain reaction (RT-PCR), mutational analysis, or methylation-specific PCR analysis. In order to clarify the specific mechanisms in the NF-κB pathway, some DLBCL-derived cell lines (probably established from pleura or lymph node) must be used. The gastrointestinal tract is one of the specific extranodal sites with special immune systems, and it is strongly affected by antigen stimulation and other cytokines compared to other organs. When the above-mentioned types of cell lines are used, conclusions that differ from those observed with intestinal DLBCL (as in the present study) might be obtained. To the best of our knowledge, there are no DLBCL cell lines derived from intestine, and we suspect that it might be difficult to demonstrate the functional status of *A20* in primary intestinal DLBCLs.

There were some discrepancies between our present FISH and IHC results, and we speculate that a FISH analysis could be a better way for determining the *A20* genetic status compared to immunohistochemical analyses. Giulino *et al.* reported the first immunohistochemical findings for A20 [14]. They observed that an *A20*-biallelic mutation case (1 case) was negative for A20 IHC, whereas cases with *A20* mutation and/or monoallelic deletion frequently retained reactivity toward A20 IHC.

Based on comprehensive gene expression analyses, DLBCLs are subclassified into GCB, non-GCB, and unclassified types, and there are significant prognostic differences between the GCB type and the non-GCB type [17]. It is thus important to classify the cells-of-or-

igin by immunohistochemistry in routine practice. In primary gastrointestinal DLBCLs, the frequency of the non-GCB type was reported to be 6-14% in the small intestine, and 57% in the colon [18, 19]. In the present series, approx. 70% of the cases were non-GCB type. This relatively higher prevalence of the non-GCB type might be due to the higher proportion of ileocecal samples in this study and/or to genetic factors, as DLBCL cases in Taiwan, regardless of tumor sites, have shown a relative high frequency (72.5%) of the non-GCB phenotype [20].

We have reported that perforation, high Performance Status (>2), and no adjuvant chemotherapy were independent poor-prognosis factors for primary intestinal DLBCL [4, 5]. In addition, tumor size >8 cm was a new independent poor-prognosis factor ($p=0.03$, 95% CI: 1.11-8.34) (Table 1). To the best of our knowledge, no study has demonstrated that the tumor size of intestinal DLBCL could be an independent predictor of prognosis. The Lugano classification does not refer to the tumor size in intestinal lymphomas [21]. Large-sized tumors might carry a higher risk of perforation, and thus large tumor size could be a poor-prognosis factor.

In conclusion, we observed that *A20* abnormality was less prevalent among intestinal DLBCLs than DLBCLs at other anatomic sites. We also observed some discordancy between gene deletion and protein expression. Although the *A20* alteration status did not affect any clinicopathological characters in this series, further studies exploring alterations of *A20* and other NF-κB components in primary intestinal DLBCL are needed.

References

1. Castro FA, Jansen L, Krilaviciute A, Katalinic A, Pulte D, Sirri E, Ressing M, Holleczek B, Luttmann S and Brenner H; GEKID Cancer Survival Working Group: Survival of patients with gastric lymphoma in Germany and in the United States. J Gastroenterol Hepatol (2015) 30: 1485–1491.

2. Yoshino T, Miyake K, Ichimura K, Mannami T, Ohara N, Hamazaki S and Akagi T: Increased incidence of follicular lymphoma in the duodenum. Am J Surg Pathol (2000) 24: 688–693.

3. Matysiak-Budnik T, Jamet P, Fabiani B, Nion-Larmurier I, Marjanovic Z and Ruskoné-Fourmestraux A; Groupe D'étude des Lymphomes Digestifs (GELD); Federation Francophone de Cancerologie Digestive (FFCD), Dijon, France: Primary intestinal B-cell lymphoma: a prospective multicentre clinical study of 91 cases. Dig Liver Dis (2013) 45: 947–952.

4. Chuang SS, Ye H, Yang SF, Huang WT, Chen HK, Hsieh PP,

5. Hwang WS, Chang KY, Lu CL and Du MQ: Perforation predicts poor prognosis in patients with primary intestinal diffuse large B-cell lymphoma. Histopathology (2008) 53: 432–440.

5. Lu YH, Chang ST, Yang SF, Weng SF, Huang WT, Hsieh PP, Hsu JD, Tsou MH and Chuang SS: Primary Intestinal Diffuse Large B-cell Lymphoma in Taiwan Showed a Relative Higher Rate of Perforation and EBV Association. Appl Immunohistochem Mol Morphol (2016) 24: 541–549.

6. Compagno M, Lim WK, Grunn A, Nandula SV, Brahmachary M, Shen Q, Bertoni F, Ponzoni M, Scandurra M, Califano A, Bhagat G, Chadburn A, Dalla-Favera R and Pasqualucci L: Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. Nature (2009) 459: 717–721.

7. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshaqiri S, Wu P, Wiesmann C, Baker R, Boone DL, Ma A, Koonin EV and Dixit VM: De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signaling. Nature (2004) 430: 694–699.

8. Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP and Ma A: Failure to Regulate TNF-Induced NF-κB and Cell Death Responses in A20-Deficient Mice. Science (2000) 289: 2350–2354.

9. Paik JH, Go H, Nam SJ, Kim TM, Heo DS, Kim CW and Jeon YK: Clinicopathologic implication of *A20/TNFAIP3* deletion in diffuse large B-cell lymphoma: an analysis according to immunohistochemical subgroups and rituximab treatment. Leuk Lymphoma (2013) 54: 1934–1941.

10. Koch P, del Valle F, Berdel WE, Willich NA, Reers B, Hiddemann W, Grothaus-Pinke B, Reinartz G, Brockmann J, Temmesfeld A, Schmitz R, Rübe C, Probst A, Jaenke G, Bodenstein H, Junker A, Pott C, Schultze J, Heinecke A, Parwaresch R and Tiemann M; German Multicenter Study Group: Primary gastrointestinal non-Hodgkin's lymphoma: I. anatomic and histologic distribution, clinical features, and survival data of 371 patients registered in the German Multicenter Study GIT NHL 01/92. J Clin Oncol (2001) 19: 3861–3873.

11. Stein H, Warnke RA, Chan WC, Jaffe ES, Chan JKC, Gatter KC and Campo E: Diffuse large B-cell lymphoma, not otherwise specified; in WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th Ed. IARC Press, Lyon (2008) pp233–237.

12. Iqbal J, Sanger WG, Horsman DE, Rosenwald A, Pickering DL, Dave B, Dave S, Xiao L, Cao K, Zhu Q, Sherman S, Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Ott G, Müller-Hermelink HK, Delabie J, Braziel RM, Jaffe ES, Campo E, Lynch JC, Connors JM, Vose JM, Armitage JO, Grogan TM, Staudt LM and Chan WC: BCL2 Translocation Defines a Unique Tumor Subset within the Germinal Center B-Cell-Like Diffuse Large B-Cell Lymphoma. Am J Pathol (2004) 165: 159–166.

13. Ando M, Sato Y, Takata K, Nomoto J, Nakamura S, Ohshima K, Takeuchi T, Orita Y, Kobayashi Y and Yoshino T: *A20 (TNFAIP3)* deletion in Epstein-Barr virus-associated lymphoproliferative disorders/lymphomas. PLOS ONE (2013) 8: e56741.

14. Giulino L, Mathew S, Ballon G, Chadburn A, Barouk S, Antonicelli G, Leoncini L and Liu YF: *A20 (TNFAIP3)* genetic alterations in EBV-associated AIDS-related lymphoma. Blood (2011) 117: 4852-4854.

15. Honma K, Tsuzuki S, Nakagawa M, Tagawa H, Nakamura S, Morishima Y and Seto M: *TNFAIP3/A20* functions as a novel tumor suppressor gene in several subtypes of non-Hodgkin lymphomas. Blood (2009) 114: 2467–2475.

16. Wolfrum S, Teupser D, Tan M, Chen KY and Breslow JL: The protective effect of A20 on an atherosclerosis in apolipoprotein

E-deficient mice is associated with reduced expression of NK-kappaB target genes. Proc Natl Acad Sci USA (2007) 104: 311–317.

17. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, Müller-Hermelink HK, Campo E, Braziel RM, Jaffe ES, Pan Z, Farinha P, Smith LM, Falini B, Banham AH, Rosenwald A, Staudt LM, Connors JM, Armitage JO and Chan WC: Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood (2004) 103: 275–282.

18. Mitchell KA, Finn WG and Owens SR: Differences in germinal centre and non-germinal center phenotype in gastric and intestinal diffuse large B-cell lymphomas. Leuk Lymphoma (2008) 49: 1717–1723.

19. Connor J and Ashton-Key M: Gastric and intestinal diffuse large B-cell lymphomas are clinically and immunophenotypically different. An immunohistochemical and clinical study. Histopathology (2007) 51: 697–703.

20. Chang ST, Chen SW, Ho CH, Kuo CC, Sakata S, Takeuchi K and Chuang SS: Immunophenotypic and genetic characteristics of diffuse large B-cell lymphoma in Taiwan. J Formos Med Assoc (2016) 115: 961–967.

21. Rohatiner A, d'Amore F, Coiffier B, Crowther D, Gospodarowicz M, Isaacson P, Lister TA, Norton A, Salem P, Shipp M and Somers R: Report on a workshop convened to discuss the pathological and staging classifications of gastrointestinal tract lymphoma. Ann Oncol (1994) 5: 397–400.