Modern Pathology (2007) 20, 460–466

www.modernpathology.org

Immunoglobulin *VH* gene analysis in gastric MALT lymphomas

Hidenori Sakuma[1], Tsuneya Nakamura[2], Naomi Uemura[3], Tsutomu Chiba[4], Toshiro Sugiyama[5], Masahiro Asaka[6], Taiji Akamatsu[7], Ryuzo Ueda[8], Tadaaki Eimoto[1], Hidemi Goto[9], Shigeo Nakamura[10] and Hiroshi Inagaki[1]

[1]*Department of Pathology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan;* [2]*Department of Endoscopy, Aichi Cancer Center Central Hospital, Nagoya, Japan;* [3]*Department of Gastroenterology, International Medical Center of Japan, Tokyo, Japan;* [4]*Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, Kyoto, Japan;* [5]*Department of Gastroenterology and Hematology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan;* [6]*Department of Gastroenterology, Hokkaido University Graduate School of Medicine, Sapporo, Japan;* [7]*Department of Endoscopy, Shinshu University Hospital, Matsumoto, Japan;* [8]*Department of Internal Medicine and Molecular Science, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan;* [9]*Department of Gastroenterology, Nagoya University Graduate School of Medicine, Nagoya, Japan and* [10]*Department of Pathology and Clinical Laboratories, Nagoya University Graduate School of Medicine, Nagoya, Japan*

The majority of gastric mucosa-associated lymphoid tissue (MALT) lymphomas are successfully treated with *Helicobacter pylori* eradication alone. However, certain subsets of these tumors are resistant to the eradication treatment. As *API2-MALT1* fusion is a feature of one of these subsets, we divided gastric MALT lymphomas into three groups: eradication-responsive and *API2-MALT1* fusion-negative (Group A), eradication-resistant and fusion-negative (Group B), and eradication-resistant and fusion-positive (Group C). To characterize further gastric MALT lymphomas, we analyzed *VH* genes, which do not change in the course of tumor progression, by extensive subcloning of the monoclonal PCR products of 45 cases. *VH3-23* and *VH3-30* were preferentially used in Group A tumors (14/23 cases, 61%) as compared with Group B (1/10 cases, 10%, $P=0.0094$) and Group C (2/12 cases, 17%, $P=0.017$). Tumors of Groups B and C used variegated *VH* fragments, and no dominant *VH* fragments were noted. Somatic mutation was detected in most of the cases. Ongoing mutation was detected in 3/45 cases (7%), when assessed according to strict criteria for a *confirmed* mutation. These findings suggest that inflammation-dependent tumors (Group A) may be derived from a highly restricted, probably *H. pylori*-associated, B cell subset and may not often progress to those that are inflammation-independent (Groups B and C). Although considered to be common in this tumor, ongoing mutation may be infrequent when assessed by strict criteria.
Modern Pathology (2007) **20,** 460–466. doi:10.1038/modpathol.3800758; published online 2 March 2007

Keywords: gastric MALT lymphoma; *helicobacter pylori*; eradication; immunoglobulin heavy chain variable genes; *API2-MALT1* fusion gene

Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) is a distinct low-grade lymphoma, which is typically localized long term at the site of origin.[1] A preexisting chronic inflammation, such as *Helicobacter pylori* gastritis, Hashimoto's thyroiditis, or Sjogren's syndrome is considered to influence significantly its development.[1] The etiological link between gastric MALT lymphoma and *H. pylori* gastritis has arisen from the fact that the majority of gastric MALT lymphomas (approximately 70% of cases) show complete regression on eradication of *H. pylori* alone.[2] *API2-MALT1* fusion, cloned from t(11;18)(q21;q21), is a gene alteration specific to MALT lymphomas.[3,4] *API2* is a member of the *IAP* (inhibitor of apoptosis) gene family, and is essential for the suppression of apoptosis.[5] *MALT1*, a novel

Correspondence: Dr H Inagaki, MD, Department of Pathology, Graduate School of Medical Sciences, Nagoya City University, Kawasumi, Mizuho-ku, Nagoya 467-8601, Japan.
E-mail: hinagaki@med.nagoya-cu.ac.jp
Received 04 November 2006; revised and accepted 05 January 2007; published online 2 March 2007

gene, may be involved in NF kappa B activation,[6] however, its biological function is not fully elucidated. Recent data have shown that *API2-MALT1* fusion leads to an increased inhibition of apoptosis, which thereby helps MALT lymphomas to survive.[7]

MALT lymphomas occur most often in the stomach, and recently, we systematically analyzed 115 gastric cases.[8] As cases positive for *API2-MALT1* fusion were totally resistant to *H. pylori* eradication, we divided the MALT lymphomas into three groups: eradication-responsive and *API2-MALT1* fusion-negative (Group A), eradication-resistant and fusion-negative (Group B), and eradication-resistant and fusion-positive (Group C).[8] Group A tumors showed superficial gastric wall involvement and a less advanced clinical stage. In contrast, those of Group B and C were often negative for *H. pylori* infection, and frequently showed a highly advanced clinical stage. A purely low-grade histology was noted in Group C tumors. Some of these features have been reported by other investigators as well.[9–11] These findings suggest that Group A tumors are considerably different from those of Groups B or C in their clinicopathological features.

The immunoglobulin heavy chain gene is formed by rearrangement of the variable (*VH*), diversity (*D*), and joining (*JH*) gene segments at the pre-B cell stage, and there are approximately 50 functional *VH* fragments that are grouped into seven structurally related families on the basis of at least 80% nucleotide sequence homology.[12,13] A biased usage of particular *VH* segments has been reported in chronic lymphoid leukemia,[14,15] splenic marginal zone lymphoma,[16] and MALT lymphomas of the salivary gland[17] and thymus.[18] Although gastric MALT lymphoma has been studied more intensively than MALT lymphomas in other sites, its *VH* gene usage has not been well characterized. Several small series have been performed,[19–22] and no unique use of *VH* genes has been reported. In this study, we analyzed *VH* gene usage, somatic mutation, and ongoing mutation status in a large series of gastric MALT lymphomas. In addition, we correlated the *VH* analysis to the three-group categorization of Groups A, B, and C.

Materials and methods

MALT Lymphoma Cases

A total of 132 cases of gastric MALT lymphoma cases were histologically reviewed according to criteria of the WHO classification for malignant lymphoma.[1] The tumor cells were immunohistochemically positive for CD20 and BCL2, but negative for CD3, CD5, CD10, CD23, and cyclin D1. The selection of cases was biased to those that showed resistance to *H. pylori* eradication. In the preliminary study, distinct monoclonal bands were not obtained in 78. Excluded were six cases with double-gene rearrangements, as detected on 8% polyacrylamide gels, and three cases with nonfunctional immunoglobulin genes as detected by sequence analysis. Consequently, 45 cases (43 biopsy and two resection specimens) were included in this study. Appropriate monoclonal bands were obtained in a similar frequency between eradication-responsive group (23/72 cases, 32%) and eradication-resistant group (22/60 cases, 37%). The study was conducted in accordance with the Declaration of Helsinki. Some of the present cases were included in our previous study.[8]

Testing for *H. pylori* infection, *H. pylori* eradication, and assessment of complete tumor regression have been described elsewhere.[8] Briefly, all tumor cases were tested for *H. pylori* infection using two or more tests including a histologic evaluation, tissue culture, a rapid urease test, a urea breath test, and a serological test for anti-*H. pylori* immunoglobulin G. In all cases, whether positive or negative for the infection, patients were administered a 2-week course of a proton pump inhibitor (lansoprazole) and a combination of antibiotics (amoxicillin, clarithromycin, and/or metronidazole). The eradication of *H. pylori* was assessed 6 weeks after completing the antibiotic therapy. Endoscopic examinations were repeated every 3 months until the lymphoma showed complete regression or was judged as resistant. Those that failed to show histological regression 9 months after the successful eradication of *H. pylori* or that had progressed during follow-up were judged to be resistant.

Detection of the API2-MALT1 Fusion Transcript

All MALT lymphoma cases were tested for the presence of the *API2-MALT1* fusion transcript by the multiplex reverse transcription (RT)-polymerase chain reaction (PCR) according to a method that we previously reported.[23] Briefly, total RNA was extracted from formalin-fixed, paraffin-embedded specimens by proteinase K digestion. RNA was subjected to first-round multiplex one-step RT-PCR, then to second-round nested multiplex PCRs (three in parallel). The final PCR products were stained with ethidium bromide, and run on 8% polyacrylamide gels. The fusion transcripts were confirmed by direct sequencing. RNA samples known to possess *API2-MALT1* fusion were used as positive controls. As an internal control for RNA quality, the ubiquitously expressed β-actin mRNA fragment was amplified. Our RT-PCR assay for the *API2-MALT1* fusion transcript using paraffin tissues has a high degree of efficacy, and is capable of 94% of the sensitivity and 100% of the specificity obtained with RT-PCR using frozen materials.[24]

DNA Extraction, *VH* Gene Amplification, and Subcloning

Genomic DNA was extracted from the formalin-fixed, paraffin-embedded tumor specimens by over-

night digestion with proteinase K. A seminested strategy was used for PCR amplification of the *VH* genes using a consensus primer for conserved framework-2 (FR2A) and consensus primers for the J region (external LJH and internal VLJH).[18,25,26] These primers have been used most commonly for *VH* gene analysis of malignant lymphoma. Subcloning of monoclonal PCR products was performed with pGEM T-easy vector (Promega, Madison, WI, USA) using DNA excised from the sharp rearrangement bands. Recombinant clones were randomly picked up and amplified, then those showing the expected insert size were sequenced using an ABI Prism Big Dye Terminator kit (Applied Biosystems, Foster City, CA, USA) on an automatic DNA sequencer. At least 11 clones were sequenced and analyzed in each lymphoma case.

Sequence Analysis

The DNA sequences were aligned to immunoglobulin heavy chain gene sequences from a well-established database, IgBLAST (http://www.ncbi. nlm.nih.gov/igblast/). *VH* gene sequences deviating more than 2% from that of the corresponding germline gene were defined as mutated.[27] For evaluation of ongoing mutation, the following definitions were used: *unconfirmed* mutation, a substitution mutation observed in only one of the *VH* gene clones from the same tumor specimen; and *confirmed* mutation, observed in more than one of the *VH* clones from the same tumor specimen.[27] Only *confirmed* mutations were considered as evidence of ongoing mutation; the *unconfirmed* mutations were instead ascribed to a Taq polymerase error.

Statistical Analysis

Statistical evaluation of data from two groups was performed using the χ^2 test, Fischer's exact test, and Student's *t*-test. All analyses were two-tailed. A probability value of $P<0.05$ for each test was regarded as statistically significant.

Results

Division of 45 MALT Lymphoma Cases into Three Groups Based on *H. pylori* Responsiveness and Detection of API2-MALT1 Fusion

Of the 45 cases of gastric MALT lymphoma, 36 were positive for *H. pylori* infection and the remaining nine were negative (Table 1). Twenty-three cases positive for the infection responded to *H. pylori* eradication therapy by tumor regression, whereas none of negative cases did. The *API2-MALT1* fusion transcript was detected in 12 cases, and all these cases were resistant to the eradication therapy. As in our previous study,[8] the lymphoma cases were divided into three groups based on their responsiveness to *H. pylori* eradication and detection of the *API2-MALT1* fusion transcript: Group A, eradication-responsive and fusion-negative ($n=23$); Group B, eradication-resistant and fusion-negative ($n=10$); and Group C, eradication-resistant and fusion-positive ($n=12$).

VH Gene Analysis in Gastric MALT Lymphoma

VH gene analysis was performed by sequencing at least 11 *VH* clones from each lymphoma, and a total of 583 clones were analyzed. Among the Group A tumors, 18 used the *VH3* family and five used *VH4*, and none of the cases used other *VH* families. Among Group B tumors, *VH3* was used in six cases, *VH4* in three, and another was used in one case. As for Group C, *VH3* was used in eight cases, *VH4* in three, and another gene family in one case (Table 1, Supplementary data). Regarding *VH* fragments, Group A tumors frequently used *VH3-23* in nine cases and *VH3-30* in five (total 14/23 cases, 61%). In contrast, Groups B and C tumors used variegated *VH* fragments, and no dominant *VH* fragments were noted. Compared with Group A tumors, Group B tumors used *VH3-23* and *VH3-30* fragments less frequently (1/10 cases, 10%), and this difference was statistically significant ($P=0.0094$, Table 2). Group C tumors used these two *VH* fragments (2/12 cases, 17%) with significantly lower frequency than did Group A tumors ($P=0.017$, Table 2). No dominant *D or* J_H fragments were found in any of the three groups (Table 1).

Group A, B, and C tumors showed germline *VH* sequence homology of $95.8\% \pm 1.07$ (mean $\pm$ s.e.), $93.9\% \pm 1.6$, and $95.8\% \pm 4.3$, respectively. There were no statistical differences between the groups. Deviation from the germline *VH* exceeding 2% (somatic mutation) was detected in 20/23 (87%), 9/10 (90%), and 11/12 (92%) cases of the respective groups, and these cases were judged to be mutated. Differences were not significant between the groups. In ongoing mutation analysis, tumors with a *confirmed*, *unconfirmed*, or negative mutation status, respectively detected in two, 15, and six cases of Group A tumors; in one, six, and three cases of Group B; and in 0, nine, and three of Group C. When assessed by strict criteria for a *confirmed* mutation, ongoing mutation was detected in 3/45 (7%) of our gastric MALT lymphoma cases.

Discussion

Gastric MALT lymphoma is not a uniform disease judging from its clinicopathological and molecular aspects. By grouping gastric MALT lymphomas into three entities (Groups A, B, and C), we have recently shown that Group A tumors (eradication-responsive and *API2-MALT1* fusion-negative) present a typical gastric MALT lymphoma subset, showing such features as superficial gastric wall involvement and

Table 1 *VH* gene analysis of gastric MALT lymphoma cases

Case	*Age*	*Sex*	*H. pylori infection*	*H. pylori eradication*	*API2-MALT1 fusion*	*Group*	*VH*	*Homology (%)*	*Somatic mutation*	*Ongoing mutation*			*D*	*JH*
										Confirmed	*Unconfirmed*	*No*		
1	44	M	+	Responsive	–	A	VH3–11	97.9	+		+		D2–15	JH5
2	61	M	+	Responsive	–	A	VH3–23	97.3	+		+		D5–12	JH3
3	45	F	+	Responsive	–	A	VH3–23	100	–			+	ND	JH4
4	71	F	+	Responsive	–	A	VH3–23	96.6	+		+		D3–9	JH4
5	75	M	+	Responsive	–	A	VH3–23	96.6	+		+		D6–19	JH3
6	57	F	+	Responsive	–	A	VH3–23	95.9	+			+	D3–10	JH5
7	64	F	+	Responsive	–	A	VH3–23	93.9	+		+		ND	JH6
8	51	M	+	Responsive	–	A	VH3–23	93.2	+		+		D2–15	JH4
9	66	M	+	Responsive	–	A	VH3–23	89.8	+			+	ND	JH5
10	63	M	+	Responsive	–	A	VH3–23	85.0	+		+		D6–13	JH5
11	65	F	+	Responsive	–	A	VH3–30	99.3	–			+	D6–19	JH4
12	53	M	+	Responsive	–	A	VH3–30	99.3	–		+		D2–2	JH6
13	62	F	+	Responsive	–	A	VH3–30	97.9	+		+		D2–15	JH4
14	52	F	+	Responsive	–	A	VH3–30	97.3	+		+		D4–17	JH3
15	70	M	+	Responsive	–	A	VH3–30	81.6	+		+		ND	JH5
16	54	F	+	Responsive	–	A	VH3–35	94.6	+		+		D5–18	JH6
17	59	M	+	Responsive	–	A	VH3–7	95.2	+		+		ND	JH4
18	58	M	+	Responsive	–	A	VH3–7	94.6	+	+	+		D3–22	JH3
19	52	M	+	Responsive	–	A	VH4–34	97.2	+		+		D6–13	JH4
20	75	M	+	Responsive	–	A	VH4–34	83.3	+			+	D1–26	JH4
21	60	F	+	Responsive	–	A	VH4–61	96.5	+		+		D3–10	JH5
22	70	M	+	Responsive	–	A	VH4–61	94.4	+	+	+		D6–13	JH5
23	53	F	+	Responsive	–	A	VH4–61	86.8	+			+	D6–13	JH4
24	37	F	+	Resistant	–	B	VH1–69	85.7	+		+		ND	JH4
25	62	M	+	Resistant	–	B	VH3–11	95.2	+		+		D5–24	JH6
26	41	F	–	Resistant	–	B	VH3–21	97.3	+			+	D3–10	JH6
27	60	M	+	Resistant	–	B	VH3–30	98.6	–		+		D2–2	JH6
28	73	F	+	Resistant	–	B	VH3–33	87.8	+		+		D3–16	JH4
29	70	F	+	Resistant	–	B	VH3–7	97.3	+	+	+		D6–19	JH4
30	62	F	+	Resistant	–	B	VH3–7	86.4	+		+		ND	JH3
31	82	M	+	Resistant	–	B	VH4–34	97.2	+		+		D3–10	JH5
32	72	M	+	Resistant	–	B	VH4–61	97.9	+			+	ND	JH5
33	16	M	+	Resistant	–	B	VH4–61	95.8	+			+	D6–13	JH4
34	43	M	–	Resistant	+	C	VH3–11	83.0	+		+		ND	JH4
35	50	M	–	Resistant	+	C	VH3–13	97.9	+			+	D3–22	JH4
36	53	M	+	Resistant	+	C	VH3–21	95.2	+			+	D4–23	JH4
37	81	M	–	Resistant	+	C	VH3–21	89.1	+		+		ND	JH4
38	63	F	+	Resistant	+	C	VH3–23	97.9	+		+		ND	JH5
39	42	M	–	Resistant	+	C	VH3–30	97.3	+		+		D5–12	JH6
40	70	F	+	Resistant	+	C	VH3–7	97.9	+		+		ND	JH6
41	70	M	–	Resistant	+	C	VH3–9	96.6	+			+	D6–19	JH4
42	50	F	–	Resistant	+	C	VH4–61	99.3	–		+		D3–10	JH5
43	64	F	+	Resistant	+	C	VH4–61	97.9	+		+		D3–10	JH5
44	78	F	–	Resistant	+	C	VH4–61	97.2	+		+		ND	JH5
45	60	F	–	Resistant	+	C	VH5–51	95.9	+		+		ND	JH3

Group A: responsive to eradication and negative for API2-MALT1 fusion; Group B: resistant to eradication and negative for the fusion; Group C: resistant to eradication and positive for the fusion; ND, not determined.

Table 2 Usage of *VH* fragments in gastric MALT lymphoma cases (Groups A, B, and C)

MALT lymphoma	*VH fragment*		P
	VH3-23 or VH3-30	*Others*	
Group A	14	9	
Group B	1	9	Group B *vs* Group A; 0.0094
Group C	2	10	Group C *vs* Group A; 0.017

Group A: responsive to eradication and negative for *API2-MALT1* fusion; Group B: resistant to eradication and negative for the fusion; Group C: resistant to eradication and positive for the fusion.

a less advanced clinical stage.[8] In contrast, Group B tumors (eradication-resistant and fusion-negative) and Group C tumors (eradication-resistant and fusion-positive) are frequently negative for *H. pylori* infection, and are often at an advanced clinical stage.[8,11] A purely low-grade histology without increased large cells is a feature of the latter.[8] To characterize further gastric MALT lymphoma, we performed *VH* gene analysis of 45 cases by extensive subcloning of monoclonal PCR products.

The most important finding of this study is that inflammation-dependent Group A tumors frequently used particular *VH* fragments (*VH3-23* and *VH3-30*) as compared with inflammation-independent Group B and C tumors. As there are approximately 50 different functional *VH* fragments in the human genome that can potentially be used,[12,13] the selective usage of the *VH* fragments detected in Group A tumors strongly suggests that these tumors may be derived from highly restricted B cell subsets. *VH3-23* and *VH3-30* have been closely associated with autoimmune disease, with the former found in patients with autoimmune thrombocytopenia,[28,29] Graves' disease,[30] and Wegener's granulomatosis,[31] and the latter in patients with systemic lupus erythematosus,[29] autoimmune thrombocytopenia,[28] and Kawasaki's disease.[32] In contrast, a biased usage of particular *VH* fragments has been reported in chronic lymphoid leukemia (*VH3-21*),[14,15] splenic marginal zone lymphoma (*VH1-2*),[16] and salivary MALT lymphoma (*VH1-69*).[17] We have reported recently that *VH3-23* and *VH3-30* have been frequently used in thymic MALT lymphoma, a tumor closely associated with autoimmune disease.[18,26] Although the antigen specificity of antibodies encoded by *VH3-23* and *VH3-30* is currently unknown, it is suggested that B cells using these two *VH* fragments, which may be associated with *H. pylori*, are preferentially selected for MALT lymphomagenesis in the stomach. It has been controversial as to whether the majority of inflammation-dependent tumors progress to inflammation-independent tumors. Our data showed that the *VH* usage pattern, which does not change in the course of tumor progression, was significantly different between tumors that were dependent on inflammation and those that were not. This finding suggests that such progression may not commonly occur, and further supports the hypothesis that *API2-MALT1* fusion-positive tumors (Group C), which are often negative for *H. pylori* infection[8,11] and resistant to eradication,[8,10] may arise independently of chronic inflammation.

Another important finding of this study is that ongoing mutation was infrequent in gastric MALT lymphoma (7% of total cases). This is in contrast to previous observations that ongoing mutation in this lymphoma may be common.[19,21,22] This discrepancy may be explained by a difference in the criteria used for determination of ongoing mutation. In the present study, ongoing mutation was assessed according to strict criteria for a *confirmed* mutation,[27] which is a substitution mutation observed in more than one of the *VH* clones from the same tumor specimen. In the previous analyses of gastric MALT lymphoma, ongoing mutation has been judged as present when a substitution mutation is observed in at least one of the *VH* gene clones from the same tumor specimen. When we similarly assessed our cases, the ongoing mutation rate was increased up to 77%. Conversely, we found that ongoing mutation was infrequent when we re-evaluated raw data of some previous reports, and used the criteria for a *confirmed* mutation. It would be difficult to determine whether an *unconfirmed* mutation is a Taq error or a true ongoing mutation. How to resolve this problem is expected to become a subject of future investigation. The majority of our gastric MALT lymphomas showed somatic mutation, which is consistent with a derivation from postgerminal center memory B cells.[33,34]

In conclusion, we showed that inflammation-dependent gastric MALT lymphomas frequently used particular *VH* fragments and that this usage was not found in inflammation-independent tumors. This finding suggests that the former tumors may be derived from a highly restricted, probably *H. pylori*-associated, B cell subset and that tumor progression from the former to the latter may not commonly occur. Ongoing mutation was not frequent when strictly assessed. However, precise standards need to be established as diagnosis of the presence of ongoing mutation is highly dependent on the type of criteria used.

Acknowledgement

This work was supported in part by grants from the Ministry of Education, Science, Sports, and Culture of Japan to HI.

References

1 Isaacson PG, Berger F, Muller-Hermelink HK, *et al.* Extranodal marginal zone B-cell lymphoma of mucosa-

associated lymphoid tissue (MALT lymphoma). In: Jaffe ES, Harris NL, Stein H, Vardiman JW (eds). World Health Organization Classification of Tumors. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissue. IARC Press: Lyon, 2001, pp 157–160.

2 Wotherspoon AC, Doglioni C, Diss TC, *et al.* Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet 1993;342: 575–577.

3 Dierlamm J, Baens M, Wlodarska I, *et al.* The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. Blood 1999;93:3601–3609.

4 Remstein ED, James CD, Kurtin PJ. Incidence and subtype specificity of API2-MALT1 fusion translocations in extranodal, nodal, and splenic marginal zone lymphomas. Am J Pathol 2000;156: 1183–1188.

5 Roy N, Deveraux QL, Takahashi R, *et al.* The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. EMBO J 1997;16:6914–6925.

6 Lucas PC, Yonezumi M, Inohara N, *et al.* Bcl10 and MALT1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF-kappa B signaling pathway. J Biol Chem 2001;276: 19012–19019.

7 Hosokawa Y, Suzuki H, Suzuki Y, *et al.* Antiapoptotic function of apoptosis inhibitor2-MALT1 fusion protein involved in t(11;18)(q21;q21) mucosa-associated lymphoid tissue lymphoma. Cancer Res 2004; 64:3452–3457.

8 Inagaki H, Nakamura T, Li C, *et al.* Gastric MALT lymphomas are divided into three groups based on responsiveness to *Helicobacter Pylori* eradication and detection of API2-MALT1 fusion. Am J Surg Pathol 2004;28:1560–1567.

9 Liu H, Ruskon-Fourmestraux A, Lavergne-Slove A, *et al.* Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to *Helicobacter pylori* eradication therapy. Lancet 2001;357:39–40.

10 Liu H, Ye H, Dogan A, *et al.* T(11;18)(q21;q21) is associated with advanced mucosa-associated lymphoid tissue lymphoma that expresses nuclear BCL10. Blood 2001;98:1182–1187.

11 Ye H, Liu H, Raderer M, *et al.* High incidence of t(11;18)(q21;q21) in *Helicobacter pylori* negative gastric MALT lymphoma. Blood 2003;101:2547–2550.

12 Rajewsky K. Clonal selection and learning in the antibody system. Nature 1996;381:751–758.

13 Cook GP, Tomlinson IM. The human immunoglobulin VH repertoire. Immunol Today 1995;16:237–242.

14 Tobin G, Thunberg U, Johnson A, *et al.* Chronic lymphocytic leukemias utilizing the V_H 3-21 gene display highly restricted Vlambda2-14 gene use and homologous CDR3s: implicating recognition of a common antigen epitope. Blood 2003;101: 4952–4957.

15 Tobin G, Thunberg U, Johnson A, *et al.* Somatically mutated Ig V(H)3-21 genes characterize a new subset of chronic lymphocytic leukemia. Blood 2002;99: 2262–2264.

16 Algara P, Mateo MS, Sanchez-Beato M, *et al.* Analysis of the IgV(H) somatic mutations in splenic marginal zone lymphoma defines a group of unmutated cases with frequent 7q deletion and adverse clinical course. Blood 2002;99:1299–1304.

17 Miklos JA, Swerdlow SH, Bahler DW. Salivary gland mucosa-associated lymphoid tissue lymphoma immunoglobulin V(H) genes show frequent use of V1-69 with distinctive CDR3 features. Blood 2000;95: 3878–3884.

18 Yoshida M, Okabe M, Eimoto T, *et al.* Immunoglobulin VH genes in thymic MALT lymphoma are biased toward a restricted repertoire and are frequently unmutated. J Pathol 2006;208:415–422.

19 Du MQ, Diss TC, Xu C, *et al.* Ongoing mutation in MALT lymphoma immunoglobulin gene suggests that antigen stimulation plays a role in the clonal expansion. Leukemia 1996;10:1190–1197.

20 Fujimori K, Shimodaira S, Akamatsu T, *et al.* Effect of *Helicobacter pylori* eradication on ongoing mutation of immunoglobulin genes in gastric MALT lymphoma. Br J Cancer 2005;92:312–319.

21 Nardini E, Rizzi S, Menard S, *et al.* Molecular phenotype distinguishes two subsets of gastric low-grade mucosa-associated lymphoid tissue lymphomas. Lab Invest 2002;82:535–541.

22 Qin Y, Greiner A, Hallas C, *et al.* Intraclonal offspring expansion of gastric low-grade MALT-type lymphoma: evidence for role of antigen-driven high-affinity mutation in lymphomagenesis. Lab Invest 1997;76: 477–485.

23 Inagaki H, Okabe M, Seto M, *et al.* API2-MALT1 fusion transcripts involved in mucosa-associated lymphoid tissue lymphoma: multiplex RT-PCR detection using formalin-fixed paraffin-embedded specimens. Am J Pathol 2001;158:699–706.

24 Okabe M, Inagaki H, Ohshima K, *et al.* API2-MALT1 fusion defines a distinctive clinicopathologic subtype in pulmonary extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. Am J Pathol 2003;162:1113–1122.

25 Conconi A, Bertoni F, Pedrinis E, *et al.* Nodal marginal zone B-cell lymphomas may arise from different subsets of marginal zone B lymphocytes. Blood 2001; 98:781–786.

26 Inagaki H, Chan JK, Ng JW, *et al.* Primary thymic extranodal marginal-zone B-cell lymphoma of mucosa-associated lymphoid tissue type exhibits distinctive clinicopathological and molecular features. Am J Pathol 2002;160:1435–1443.

27 Lossos IS, Okada CY, Tibshirani R, *et al.* Molecular analysis of immunoglobulin genes in diffuse large B-cell lymphomas. Blood 2000;95:1797–1803.

28 Hoffmann M, Uttenreuther-Fischer MM, Lerch H, *et al.* IVIG-bound IgG and IgM cloned by phage display from a healthy individual reveal the same restricted germline gene origin as in autoimmune thrombocytopenia. Clin Exp Immunol 2000;121:37–46.

29 Osei A, Uttenreuther-Fischer MM, Lerch H, *et al.* Restricted V_H3 gene usage in phage-displayed Fab that are selected by intravenous immunoglobulin. Arthritis Rheum 2000;43:2722–2732.

30 Shin EK, Akamizu T, Matsuda F, *et al.* Variable regions of Ig heavy chain genes encoding antithyrotropin receptor antibodies of patients with Graves' disease. J Immunol 1994;152:1485–1492.

31 Sibilia J, Benlagha K, Vanhille P, *et al.* Structural analysis of human antibodies to proteinase 3 from patients with Wegener granulomatosis. J Immunol 1997;159:712–719.

32 Leucht S, Uttenreuther-Fischer MM, Gaedicke G, *et al.* The B cell superantigen-like interaction of intravenous immunoglobin (IVIG) with Fab fragments of V(H) 3-23 and 3-30/3-30.5 germline gene origin cloned from a patient with Kawasaki disease is enhanced after IVIG therapy. Clin Immunol 2001;99:18–29.

33 Kuppers R, Klein U, Hansmann ML, *et al.* Cellular origin of human B-cell lymphomas. N Engl J Med 1999;341:1520–1529.

34 Spencer JO, Dunn-Walters DK. Somatic hypermutation and B-cell malignancies. J Pathol 1999;187: 158–163.

Supplementary Information accompanies the paper on the Modern Pathology website (http://www.nature.com/modpathol)