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SHORT REPORT

High frequency of t(11;18) in gastric mucosa-associated lymphoid tissue lymphomas in Taiwan, including one patient with high-grade transformation

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Summary. t(11;18)(q21;q21), the most frequent chromosomal aberration of mucosa-associated lymphoid tissue (MALT) lymphoma, occurs in 30% of gastric patients. Although the translocation is often associated with an 'aggressive' course, it has not been described in transformed MALT lymphomas. We screened 15 gastric MALT lymphomas [three with concurrent or subsequent high-grade transformation and 11 diffuse large B-cell lymphomas (DLBCLs)] in Chinese patients for t(11;18). t(11;18) was found in 9/15 (60%) MALT lymphomas, but not in any DLBCLs. One patient, with subsequent high-grade transformation, showed the translocation in low- and high-grade lesions. t(11;18) was frequent in Chinese gastric MALT lymphomas and unusually one transformed lymphoma carried the translocation.

Keywords: high-grade transformation, MALT lymphoma, p53, RT-PCR, t(11;18).

The development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma is a multistage process. The neoplastic clone arises from acquired MALT induced by *Helicobacter pylori* infection. The tumour cells initially infiltrate around the reactive lymphoid follicles in the mucosal layer. Subsequently, they expand to form a diffuse infiltrate, invade deeper layers of the gastric wall and disseminate to local lymph nodes or remote sites. MALT lymphoma may transform into high-grade lymphoma with features ranging from a focal high-grade component to diffuse large B-cell lymphoma (DLBCL).

t(11;18)(q21;q21) is a chromosomal aberration specifically associated with MALT lymphoma (Auer *et al*, 1997; Ott *et al*, 1997). The translocation fuses the amino terminal of the *API2* gene to the carboxyl terminal of the *MALT1* gene. In gastric MALT lymphoma, t(11;18) occurs in around 30% of patients (Baens *et al*, 2000; Dierlamm *et al*, 2000; Inagaki *et al*, 2001) and translocation-positive tumours do not respond to *H. pylori* eradication (Liu *et al*, 2001a, 2002). The translocation has not been described in transformed MALT lymphoma or mucosal DLBCL in Caucasian and Japanese patients despite t(11;18) being more frequent in the advanced cases (Rosenwald *et al*, 1999; Baens *et al*, 2000; Inagaki *et al*, 2001; Liu *et al*, 2001b).

In view of the value of t(11;18) in clinical management of patients with gastric MALT lymphoma, it is important to examine the frequency of the translocation in patients of other ethnic backgrounds. We studied primary gastric lymphomas from Taiwan and found that the t(11;18) occurred in 60% of gastric MALT lymphomas and was also present in one transformed MALT lymphoma.

MATERIALS AND METHODS

Materials. Paraffin blocks of 15 gastric MALT lymphomas, including three patients with concurrent or subsequent high-grade transformation and 11 primary gastric DLBCLs, were retrieved from the Chi-Mei Medical Centre in southern Taiwan. They were staged with ultrasonography, computerized tomography scan and/or magnetic resonance imaging, and bone marrow biopsy according to the Ann Arbor system (Musshoff, 1977).

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Immunohistochemistry. Immunophenotyping was performed for all patients. Selected cases were immunostained with anti-p53 protein (Serotec, Oxford, UK), Ki-67 and *H. pylori* antibodies (Dako, Glostrup, Denmark).

Reverse-transcription polymerase chain reaction (RT-PCR) for detection of t(11;18). RNA extraction, cDNA synthesis, and PCR amplification were carried out as described (Liu *et al*, 2002). Reverse-transcription was carried out using the SuperScript™ Preamplification System (Life Technologies, Scotland, UK) with a mixture of gene-specific primers, including one pair of glucose-6-phosphate dehydrogenase (G6PD) primers, which were specially designed for formalin-fixed paraffin-embedded tissues. Three sets of PCR primers with a common sense primer covering 93% of the known breakpoints on the API2 gene (5′-GGAAGAGGAGAGA-GAAAGAGCA, API2 cDNA nucleic acids 2008–2029, GenBank Accession No. NM_001165) and three antisense primers (5′-CCAAGACTGCCTTTGACTCT, MALT1 cDNA nucleic acids 563–582; 5′-GGATTCAGAGACGCCATCAA, MALT1 cDNA nucleic acids 820–839 and 5′-CA-AAGGCTGGTCAGTTGTTT, MALT1 cDNA nucleic acids 1162–1181, GenBank Accession No AF130356) targeting all four breakpoints on the MALT1 gene were used for PCR of the API2-MALT1 fusion transcript as previously described (Liu *et al*, 2002). G6PD was amplified in parallel as control. PCR was performed separately with each primer set, at least in duplicate.

RT-PCR products of the API2-MALT1 transcript in all cases were either directly sequenced or cloned into a vector (the TOPO TA Cloning® Kit; Invitrogen, Paisley, UK) and sequenced with vector primers using dRhodamine dye terminators on an ABI Prism 377 sequencer (PE Applied Biosystems, Foster City, CA, USA).

Clonality analysis. B-cell clonality was studied by PCR of both the rearranged immunoglobulin (Ig) heavy and light chain genes as described (Diss *et al*, 1994, 2002).

RESULTS AND DISCUSSION

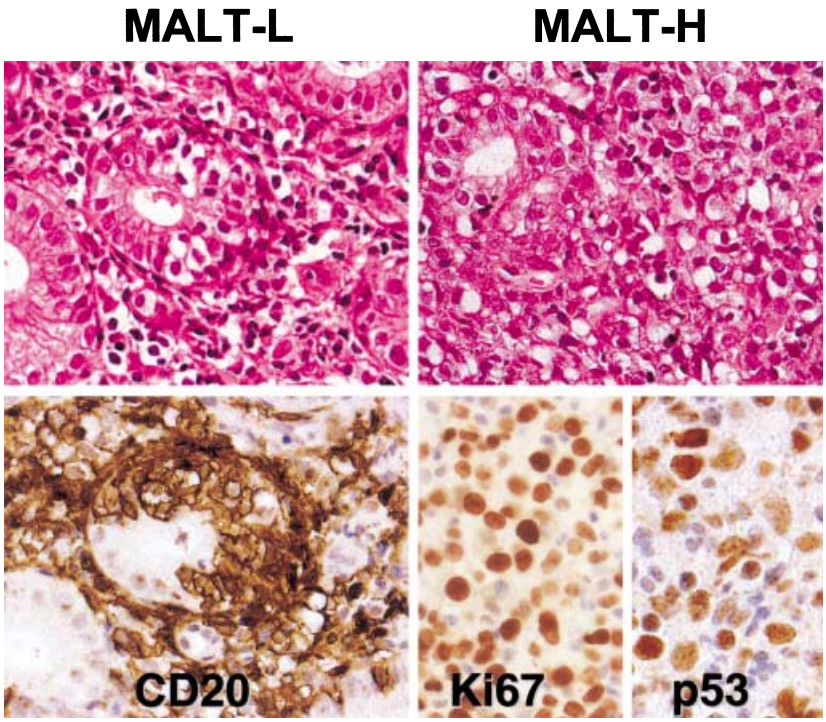
The clinicopathological and molecular data of the gastric MALT lymphoma patients are summarized in Table I. All 15 MALT lymphomas except patient 3 were positive for *H. pylori* by light microscopy, immunohistochemistry and/or serology. Of the 14 patients in which clinical staging was available, nine were at stage IE and the remaining five were at stage IIE. On histological review, 12 patients were classified as pure MALT lymphoma and two patients (patients 13, 14) as MALT lymphoma with co-existing focal high-grade components. The remaining patient (patient 15) was initially diagnosed with an *H. pylori*-associated gastric ulcer (0·5 cm in dimension by endoscopy) and the patient did not receive *H. pylori* eradication therapy. Two years later, the patient presented with a large ulcerative tumour of 13 cm occupying the entire gastric antrum extending to the body. The patient received two courses of chemotherapy [cyclophosphamide, epirubicin, vincristine (oncovin) and prednisolone] but died of lymphoma 3 months later. Histological review of the biopsies showed MALT lymphoma in the first biopsy (patient 15a) and transformed MALT lymphoma without a low-grade component in the second biopsy (patient 15b) (Fig 1).

Table I. Summary of clinicopathological and molecular data of MALT lymphomas.

Patient	Age (years)	Sex	Specimen type	HP	Diagnosis	Stage	t(11;18)	Breakpoint *API2/MALT1**	P53 staining
1	82	M	Bx	+	MALT-L	IE	+	2048/814	–
2	68	F	Bx	+	MALT-L	IE	+	2048/814	–
3	38	F	Surg	–	MALT-L	IIE2	+	2048/814	+
4a	81	M	Bx	+	MALT-L	IIE1	+	2048/814	–
4b			Bx	+	MALT-L	IIE1	+	2048/814	–
5	56	M	Bx	+	MALT-L	IE	+	2048/814	–
6	73	F	Surg	+	MALT-L	IE	+	2048/814	–
7	70	M	Bx	+	MALT-L	IE	+	2048/814	–
8	61	F	Bx	+	MALT-L	NA	–	–	–
9	72	M	Bx	+	MALT-L	IIE1	–	–	–
10	60	F	Bx	+	MALT-L	IIE2	–	–	–
11	60	M	Bx	+	MALT-L	IE	–	–	–
12	78	M	Bx	+	MALT-L	NA	–	–	ND
13a	79	M	Surg	+	MALT-L	IIE1	+	2048/814	–
13b			Surg	+	MALT-H	IIE1	–	–	+
14	72	M	Bx	+	MALT-L & H	IE	–	–	–
15a	72	F	Bx	+	MALT-L	IE	+	2048/814	Unsatis
15b			Bx	+	MALT-H	IE	+	2048/814	+

*The number of nucleotides was according to the *API2* (NM_001165) and *MALT1* (AF130356) sequences.

Bx, biopsy; HP, *Helicobacter pylori*; MALT-L, low-grade MALT lymphoma; MALT-L & H, MALT-L with co-existing high-grade component; MALT-H, transformed MALT lymphoma without low-grade component; NA, not available; ND, not done; Surg, gastrectomy; Unsatis, unsatisfactory.



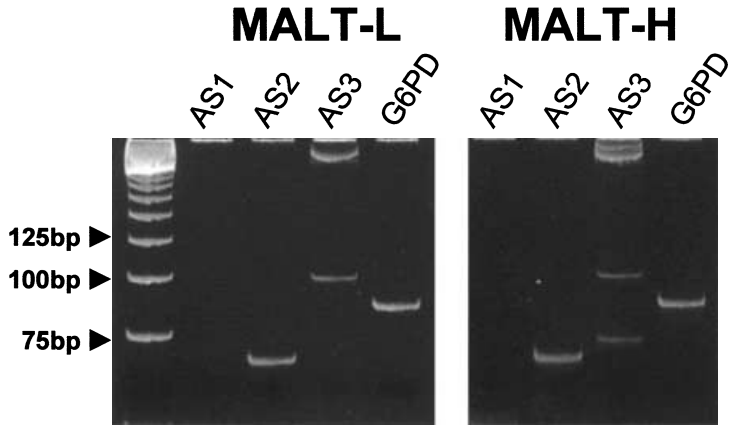


Fig 1. Histological and molecular study of Patient 15. Upper panel: biopsy (15a) shows a low-grade MALT lymphoma (MALT-L) with CD20-expressing small lymphoma cells infiltrating the gastric gland. Biopsy (15b) displays the transformed MALT lymphoma (MALT-H) in which tumour cells are large with nuclear expression of Ki67 and p53 (original magnification, ×400). Lower panel: RT-PCR of the API2-MALT1 fusion transcript shows positive products in both MALT-L and MALT-H. With the antisense (AS) 2 primer set, both lesions yielded a 67 bp product. With AS3 primer set, MALT-L showed a 100 bp product while MALT-H displayed an additional 73 bp product, which was due to alternative splicing.

Nine of the 15 (60%) gastric MALT lymphomas, but none of the 11 primary DLBCLs, showed a positive RT-PCR for API2-MALT1. In each positive patient, the PCR product was confirmed to be the API2-MALT1 fusion transcript by sequencing. Interestingly, the API2-MALT1 fusion transcript was found in two of the three MALT lymphomas with concurrent or subsequent high-grade transformation. In patient 15, the same API2-MALT1 fusion transcript was detected in both the initial MALT lymphoma and subsequently transformed tumour that did not contain any low-grade component (Fig 1). In patient 13, the fusion transcript was detected from whole tumour sections but not from microdissected high-grade cells.

In an attempt to indirectly confirm the presence of t(11;18) in the high-grade lesion of the above patients, we performed Ig heavy and light chain PCR in order to establish the clonal relationship between the low- and high-grade components. In both patients, these PCRs failed to show a dominant band.

Nonetheless, in patient 15, the presence of t(11;18) was convincingly demonstrated in both MALT and the transformed large cell lymphoma. Thus, t(11;18)-positive MALT lymphoma may undergo high-grade transformation. The reason why t(11;18)-positive MALT lymphoma underwent high-grade transformation in this Chinese patient but has not been found in Caucasian patients is unclear. The incidence of t(11;18) in gastric MALT lymphoma of Chinese patients from Taiwan is relatively higher than that of the West. It remains to be investigated whether these findings represent a real difference in the genetics of MALT

lymphoma between Chinese and Caucasian patients or are due to sample variations.

To further examine the mechanism underlying high-grade transformation of gastric MALT lymphoma, we carried out p53 immunohistochemistry as its inactivation has been shown to be associated with MALT lymphoma transformation. Among the 14 patients examined, three patients, including two transformed MALT lymphomas (patients 13, 15), showed p53 nuclear staining. In patient 13, p53 staining was only found in the transformed MALT lymphoma but not in the low-grade lesion. While in patient 15, strong staining was seen in the nuclei of large neoplastic cells of the transformed MALT lymphoma (Fig 1), the staining was unsatisfactory for this MALT lymphoma. The finding of both t(11;18) and p53 inactivation in this patient may explain the aggressive clinicopathological behaviour and highlights that patients with t(11;18)-positive MALT lymphoma need urgent treatment.

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