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Case report

Helicobacter pylori-negative / *API2-MALT1* translocation-negative low-grade MALT lymphoma

Koji Nozaki[1], Sachiyo Nomura[1], Nobuyuki Shimizu[1], Naoki Hiki[1], Nao Yoshizawa[1], Susumu Aikou[1], Keisuke Kubota[1], Hirokazu Yamaguchi[1], Hanzo Kurosaka[1], Aya Shinozaki[2], Ken-ichi Mafune[1], Masashi Fukayama[2], and Michio Kaminishi[1]

[1]Department of Gastrointestinal Surgery, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
[2]Department of Pathology, The University of Tokyo, Tokyo, Japan

Abstract
A 71-year-old man with a *Helicobacter pylori* infection-negative and *API2-MALT1* translocation-negative extranodal marginal-zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type of the stomach has been followed conservatively for over 5 years. The lesion has shown no major morphological changes or malignant progression into a diffuse large-cell type during the time course. The absence of genetic translocation of *API2-MALT1* was confirmed with fluorescence in situ hybridization (FISH). The prognosis of *H. pylori*-negative and *API2-MALT1* translocation-negative low-grade MALT lymphoma is unknown, and a standard treatment for such lymphoma has yet to be defined. The case of MALT lymphoma negative for both of the above factors that we report has shown no obvious rapid progression or malignant change over the long-term course. Although curative operation and/or chemoradiotherapy should still be discussed as the treatment of choice, the treatment of this type of lymphoma must be carefully determined on a case-by-case basis, according to its biological status and prognosis.

Key words MALT lymphoma · *Helicobacter pylori* · *API2-MALT1* translocation

Introduction

Gastric extranodal marginal-zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) type [1,2] is strongly related to *Helicobacter pylori* infection, which has been documented as being involved in up to 90% of patients with low-grade gastric MALT lymphoma. Infection with *H. pylori* can provide a T-cell-mediated antigenic stimulus that induces the sustained growth of MALT tumors, and the eradication of *H. pylori* (currently recommended as the treatment of choice for *H. pylori*-positive gastric MALT lymphoma) may induce a complete remission in localized cases [3].

The t(11;18)(q21;q21) translocation [4–13], resulting in a chimeric transcript encoding an *API2-MALT1* fusion product, has been clarified as the most frequent chromosome aberration in MALT lymphomas, whose action may lead to an increased inhibition of apoptosis, therefore conferring a survival advantage on tumor cells. It is considered that gastric MALT lymphomas that do not show regression in response to *H. pylori* eradication usually express the *API2-MALT1* chimeric transcript mediated by the t(11;18)(q21;q21) translocation, and that this gene aberration thus serves as a reliable predictive marker for responsiveness to anti-*H. pylori* treatment [4].

MALT lymphomas comprise 7.6% of all non-Hodgkin's lymphomas and represent one of the most common non-Hodgkin's lymphomas [14]. In a previous report, about 89% of MALT lymphoma cases were *H. pylori*-positive and 11% were *H. pylori*-negative, while about 17% of MALT lymphoma cases were *API2-MALT1* chimeric transcript-positive and 83% were *API2-MALT1* chimeric transcript-negative [15]. Furthermore, 4 cases (7.5%) of *H. pylori*-positive and *API2-MALT1*-positive MALT lymphomas and 1 case (1.9%) of an *H. pylori*-negative and *API2-MALT1*-negative MALT lymphoma were included in this report [15].

Histologically, *API2-MALT1*-positive lymphomas featured dense lymphocytic infiltration, predominantly in the submucosa, and a monotonous proliferation of centrocyte-like cells, infrequent lymphoepithelial lesions, and, often, few reactive components such as plasma cells and eosinophils. These findings were also seen in three patients with *API2-MALT1*-negative lymphomas who did not respond to treatment. However, marked plasmacytoid differentiation of the tumor cells, simulating an extramedullary plasmacytoma, was also

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observed in two patients with *API2-MALT1*-negative lymphomas who responded to treatment [16]. In the clinical course of patients with the *API2-MALT1* chimeric transcript-positive lymphomas, progression of clinical stage, high-grade transformation, and chromosomal aberrations were not apparent, indicating their high biological stability [17].

As for patients with *H. pylori*-negative and *API2-MALT1* chimeric transcript-negative lymphomas, information is so scarce that their prognosis remains unknown. We report here a patient with *H. pylori*-negative and *API2-MALT1* translocation-negative low-grade MALT lymphoma of the stomach, who has been followed conservatively for over 5 years.

Case report

A 71-year-old man with no specific symptoms underwent upper gastrointestinal (GI) tract radiography during a routine medical examination for screening, and an ulcerative lesion with a gathering of thickened folds was detected. Pathological study of the biopsy specimen with H&E and immunohistochemical staining led to a diagnosis of the lesion as low-grade mucosa-associated lymphoid tissue (MALT) lymphoma with out the presence of *H. pylori*. The absence of *H. pylori* infection was also confirmed by the ^{13}C urease breath test (UBT), in addition to repeated immunohistological studies and *H. pylori* culture studies from biopsy specimens.

Upper GI tract radiography examination demonstrated an ulcerative lesion with a gathering of folds on the posterior wall in the corpus of the stomach (Fig. 1A,B). Endoscopic ultrasonography (EUS) imaging showed the lesion to have a maximum thickness of about 2 cm, extending over all the layers of the stomach, including the mucosa, submucosa, and muscle layer. Computed tomography (CT) scanning showed the lesion covering almost all the stomach layers, but with no obvious signs of local or distant metastasis. These findings matched those from a screening with magnetic resonance imaging (MRI). Systemic whole-body screening with scintigraphy, using ^{67}Ga-citrate 74 MBq, demonstrated no distant metastasis. Finally, the patient was diagnosed as having advanced (deeply invading to the gastric mucosa but with no lymph-node metastasis) low-grade MALT lymphoma, with no involvement of *H. pylori*.

The lymphoma (Fig. 2A) was comprised of small cells with monoclonal proliferation positive for the lambda (λ) chain of gamma (γ) globulin. Immunoreactive kappa (κ) chain-positive cells were not dominant. Dutcher bodies and lymphoepithelial lesions (LELs) (Fig. 2B) were also observed. The lesion also contained Russell bodies (Fig. 2C) with accumulation of denatured γ globulin in the plasma cells. Most of the infiltrating lymphoid cells showed positive immunostaining for the λ chain (Fig. 2D), CD45 (Fig. 2E), CD20 (Fig. 2F), and CD79a, whereas T cells positive for CD3 or CD45R were very few.

All results were carefully explained to the patient, a considerable number of options for medical treatment were explained and proposed, and a curative surgical operation or systemic chemotherapy was strongly recommended. However, the patient refused to undergo any clinical therapy because he had no specific symptoms, pain, or discomfort. The lesion was *H. pylori*-negative, but triple combination therapy with lansoprazole (LPZ), clarithromycin (CAM), and amoxicillin (AMPC) was tentatively performed for 1 week, with the patient's consent. A regular follow-up

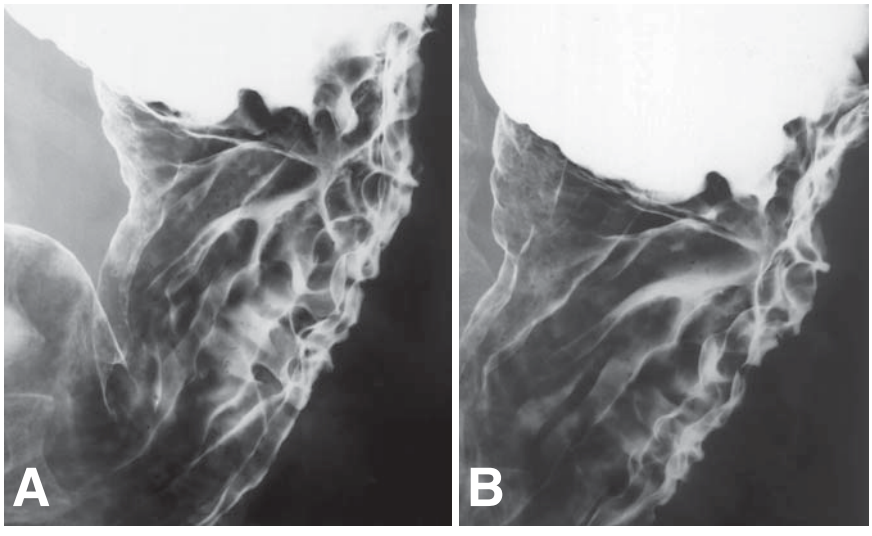

Fig. 1A,B. An ulcerative lesion with a gathering of folds on the posterior wall in the corpus of the stomach, demonstrated by upper gastrointestinal (GI) tract radiography examination

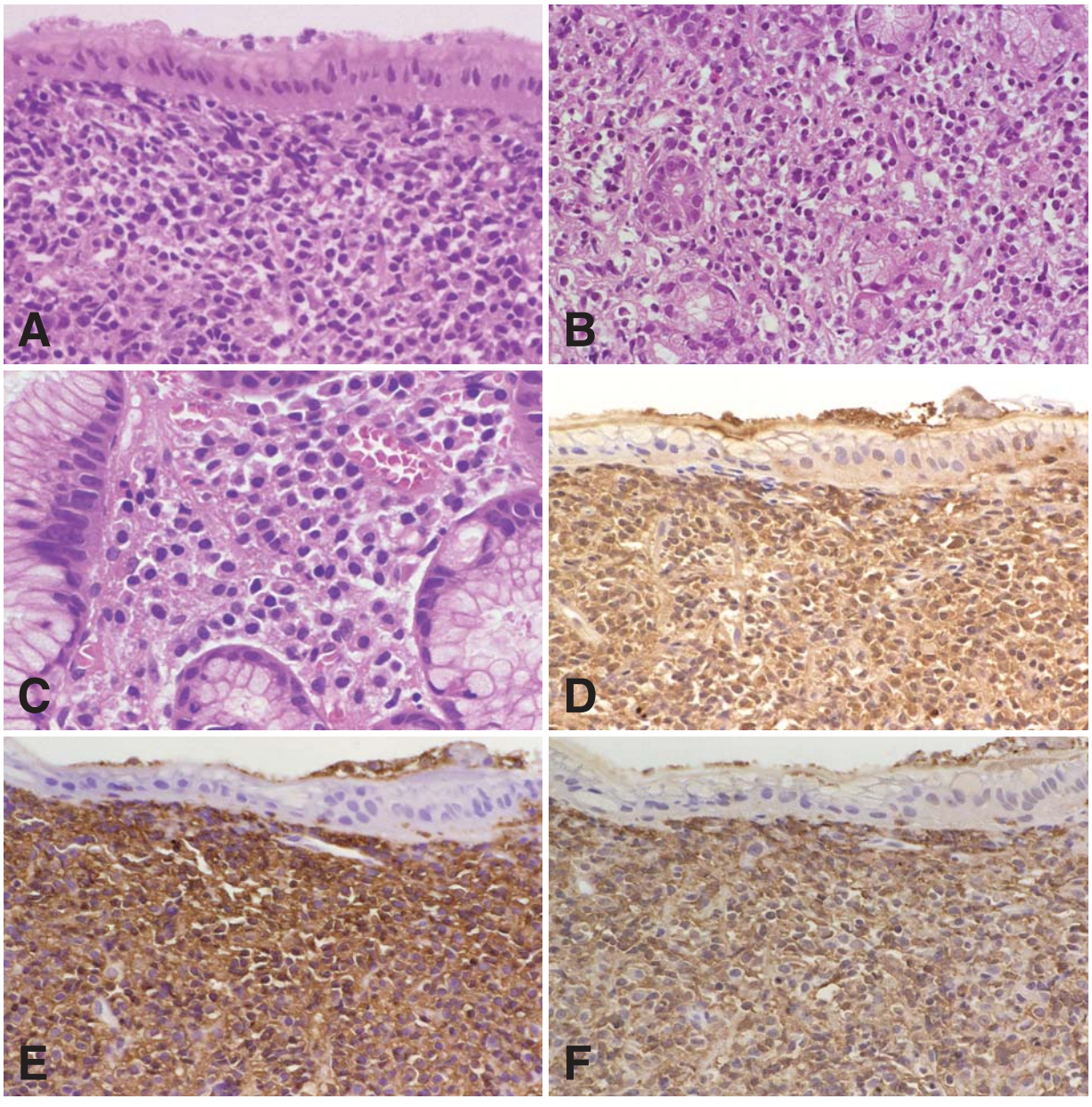

Fig. 2. A Low-grade mucosa-associated lymphoid tissue (MALT) lymphoma cells. **B** A lymphoepithelial lesion (LEL) was evident in the MALT lymphoma tissue. **C** Russell bodies were included in the MALT lymphoma tissue. **D** The lymphoma consisted of small cells with monoclonal proliferation positive for the lambda (λ) chain of gamma (γ) globulin. In contrast, gastric epithelial cells were negative for λ-chain immunostaining. **E** Lymphoma cells were CD45-positive and epithelial cells were CD45-negative. **F** Lymphoma cells were positive for CD20 immunostaining. **A** and **B** H&E, ×100; **C** H&E, ×200; **D** Immunohistochemistry for lambda (λ) chain, ×100; **E** Immunohistochemistry for CD45, ×100; **F** Immunohistochemistry for CD20, ×100

course of gastrointestinal endoscopy was initiated, and carried out at 1-year intervals.

Genetic translocation of *API2-MALT1* from the biopsy samples was examined using fluorescence in-situ hybridization (FISH). An LSI MALT1 (18q21) dual-color, break-apart rearrangement probe (Vysis, Downers Grove, IL, USA), comprised of a mixture of two FISH DNA probes, was used for the study. A 460-kb probe labeled in orange flanked the 5′ side of the *MALT1* gene, and a 660-kb probe labeled in green flanked the 3′ side of the *MALT1* gene. There was a 65-kb gap between the two probes. The known breakpoints exist in introns 2, 3, and 8 of the *MALT1* gene. If a cell lacks the t(18q21) translocation in the *MALT1* gene region, two orange and green fusion signal patterns, reflecting two intact copies of *MALT1*, will be

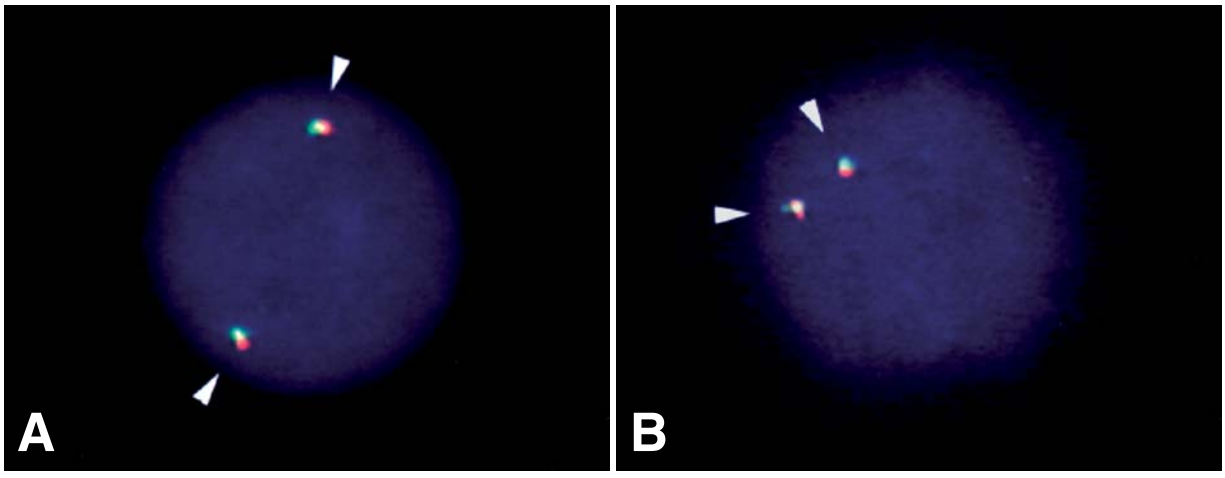

Fig. 3. Genetic translocation analysis of *API2-MALT1* from biopsy samples, using fluorescence in situ hybridization (FISH). An LSI MALT1 (18q21) dual-color, break-apart rearrangement probe (Vysis), comprised of a mixture of two FISH DNA probes, as used for the study. A 460-kb probe labeled in orange flanks the 5′ side of the *MALT1* gene, and a 660-kb probe, labeled in green, flanks the 3′ side of the *MALT1* gene. **A,B** Cells showing two *orange and green fusion signal patterns* (*arrowheads*), reflecting two intact copies of *MALT1*. The genetic translocation proved to be absent

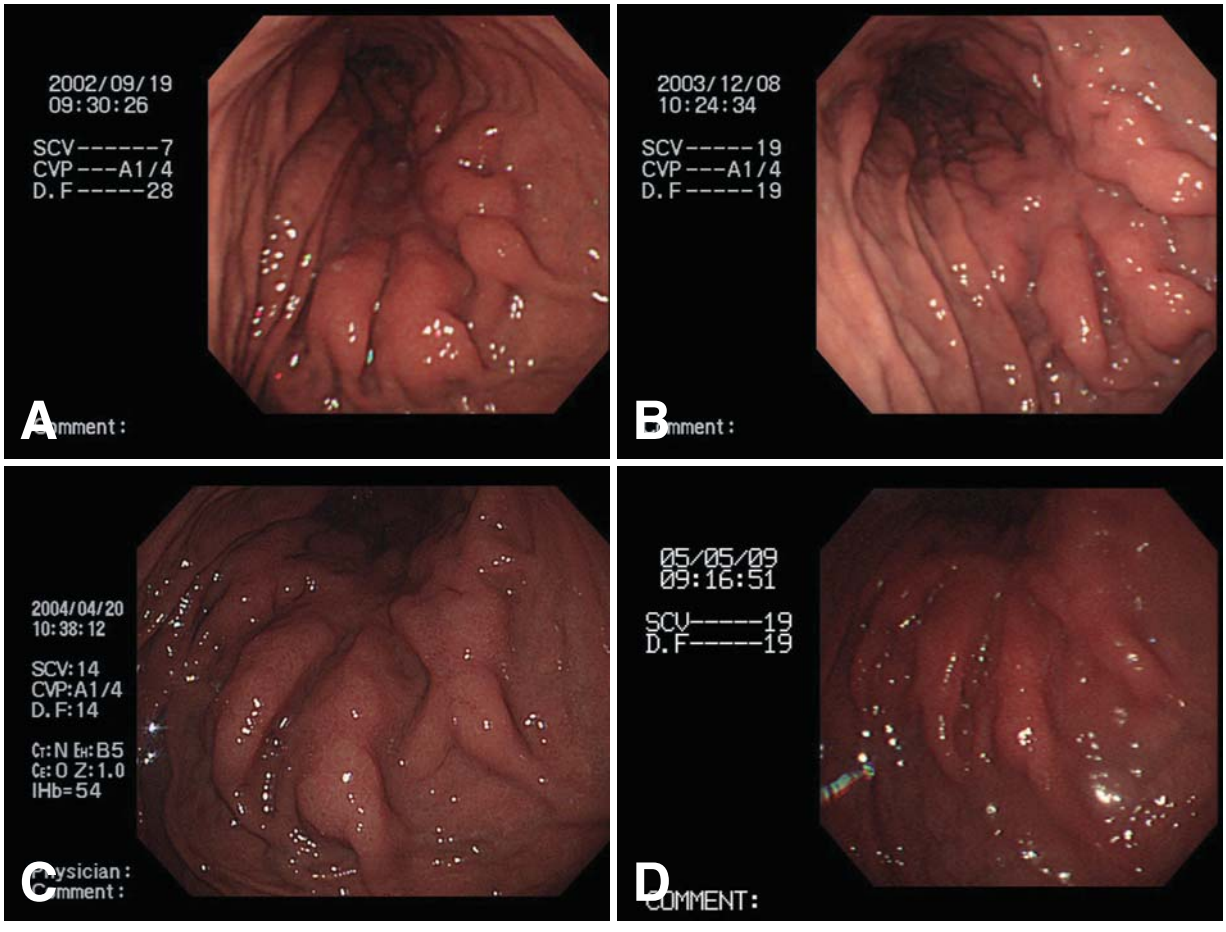

Fig. 4A–D. Annual endoscopic findings. The lesion of low-grade MALT lymphoma showed little or no change in morphology and size

demonstrated. If a cell contains the t(18q21) translocation, one green, one orange, and one fusion signal pattern will be demonstrated. This case, showed two orange and green fusion signal patterns, reflecting two intact copies of MALT1. The same results were confirmed in all biopsy specimens, and the genetic translocation proved to be absent (Fig. 3A,B).

Endoscopic findings revealed almost no change in the patient's low-grade MALT lymphoma (Fig. 4A–D), nor did pathologic findings show any change. Follow-up CT screenings also demonstrated the lesion as showing no change, with no obvious metastasis to the lymph nodes, or distant metastasis.

Discussion

The prognosis of *H. pylori*-negative and *API2-MALT1* translocation-negative low-grade MALT lymphoma is unknown, and a standard treatment has yet to be defined.

The majority of low-grade MALT lymphomas are *H. pylori*-positive and *API2-MALT1* translocation-negative, and are quite sensitive to *H. pylori* eradication. However, some of them can rapidly develop malignant changes, progressing to the high-grade large-cell type; therefore, very careful attention is required to clarify whether a remission due to *H. pylori*-eradication is lasting. In contrast, approximately 10% of gastric low-grade B-cell lymphomas of the mucosa-associated lymphoid tissue (MALT) type are unresponsive to *H. pylori* eradication treatment, and many of them contain an *API2-MALT1* chimeric transcript mediated by the t(11;18)(q21;q21) translocation. Some MALT lymphomas can also be associated with the t(1;14)(p22;q32) translocation [18] or the t(14;18)(q32;q21) translocation [19]. The t(11;18)(q21;q21) translocation fuses the N-terminus of the *API2* gene and the C-terminus of the *MALT1* gene, generating a functional *API2-MALT1* product. The t(1;14)(p22;q32) translocation or the t(14;18)(q32;q21) translocation bring the *BCL10* or *MALT1* genes, respectively, to the Immunoglobulin Heavy (IGH) locus and deregulate their expression. The oncogenic activity of these chromosomal translocations is linked by the physiological role of *BCL10* and *MALT1* in antigen receptor-mediated nuclear factor (NF) kappaB activation, and may confer a survival advantage to MALT B-cells, through anti-apoptotic or proliferative transcriptional signals.

The presence of the t(11;18)(q21;q21) translocation-induced *API2-MALT1* chimeric transcript can be considered a reliably predictive marker for nonresponsiveness to *H. pylori* eradication treatment in patients with low-grade gastric MALT lymphoma [3–10]. Liu et al. [20] documented that they detected the *API2-MALT1* transcript in 75% of patients who were nonresponsive to antibiotic therapy, but they did not detect the transcript in responsive patients. They concluded that most *H. pylori*-associated gastric MALT lymphomas that do not respond to antibiotic therapy are associated with the t(11;18)(q21;q21) translocation [20]. In another report [21], the t(11;18)(q21;q21) translocation was positive in 43 of 63 gastric MALT lymphomas that were nonresponsive to *H. pylori* eradication, and, in 48 patients who showed complete regression, 2 who were positive for the t(11;18)(q21;q21) translocation showed a relapse of lymphoma, in the absence of *H. pylori* reinfection. *API2* is expressed in cells that correspond to mature B cells, whereas *MALT1* mRNA is detectable in pre-B cells, mature B cells, and plasma cells, so that the fusion of *MALT1* to *API2* mediated by t(11;18)(q21;q21) is considered to result in an increased inhibition of germinal center B-cell apoptosis and the subsequent development of MALT lymphomas [22].

Malignant lymphomas can be also seen in patients with hepatitis B virus (HBV) [23] or hepatitis C virus (HCV) [24] infection. HBV is a circular DNA virus with a 3.2-kb partially double-stranded DNA genome, and it can insert several viral genomes into a host cell. HCV is a non-retroviral RNA virus, which can exert its carcinogenic influence through chronic liver damage, consequent cellular regeneration, and stimulation of humoral and cellular immune response. Both acute and chronic HCV infection cause an increase in mutation frequency in the immunoglobulin heavy chain, *BCL-6*, *p53*, and beta-catenin genes. HCV can also induce transformation of the *ras* gene, and the inhibition of apoptosis can be induced by the HCV core protein. HCV is also reported to be well-associated with the formation of B-cell lymphoma. The present patient had no previous history of HBV or HCV infection.

In the present patient, the *H. pylori*-negative and *API2-MALT1* translocation-negative gastric MALT lymphoma has shown no apparent changes in the its morphology over a long-term observation period of more than 5 years. Such an advanced MALT lymphoma suggests a new concept regarding the malignant potential of *H. pylori*-negative MALT lymphomas. Although curative surgery or systemic chemoradiotherapy should still be considered, the apparent absence of malignant changes in the lymphoma reported here suggests that the prognosis of these lymphomas should be discussed and carefully evaluated, and surgical indications should be determined on a case-by-case basis.

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