Characteristics of gastric B-cell lymphoma of mucosa-associated lymphoid tissue type involving multiple organs

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Introduction

The stomach is one of the most common organs in which mucosa-associated lymphoid tissue (MALT) lymphoma develops. It is well established that Helicobacter pylori infection has a major role in the development of gastric MALT lymphoma.1 Indeed, since the first report, of Wotherspoon et al.,2 many clinical trials of eradication therapy for H. pylori have demonstrated regression in 70% to 80% of patients with gastric MALT lymphoma.3–6 On the other hand, recent molecular analysis studies have revealed that t(11;18)(q21;q21), a translocation between API2 at 11q21 and MALT1 at 18q21, is present in approximately half of the patients with low-grade MALT lymphoma, suggesting etiologic roles of the API2-MALT1 fusion transcript in the development of low-grade MALT lymphoma.7–11 The precise role of the API2-MALT1 fusion transcript in the development of gastric MALT lymphoma, however, remains unclear.

Gastric MALT lymphoma is characterized by an indolent clinical course, and generally remains localized within the stomach for a prolonged period.12 There are occasional cases, however, of gastric MALT lymphoma with multiple lesions within the stomach. Moreover, cases of gastric MALT lymphoma with simultaneous development in different organs have also been reported.13–20 In such cases, two possibilities are considered; one is that the tumor cells disseminate from an original site to the other sites or organs, or, alternatively, the tumor cells originate from different sites independently. Previous reports indicate the possible dissemination of tumor cells to other organs.15,16,18

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whereas cases originating from different clones in different organs have also been reported. MALT lymphoma involving multiple organs is not well characterized. In this context, the pathophysiologic roles of the API2-MALT1 fusion transcript and *H. pylori* infection in gastric MALT lymphoma with multiple organ involvement are also unclear. In the present study, therefore, to further clarify the characteristics of gastric MALT lymphoma with multiple organ involvement, we examined the *H. pylori* infection status and the effects of eradication therapy, and performed genetic analysis, including an analysis of t(11;18)(q21;q21) and the clonality of tumor cells.

**Patients and methods**

**Patients**

There were 63 patients with extranodal gastric low-grade MALT lymphoma at Kyoto University Hospital and affiliated hospitals between 1995 and 2002. The diagnosis was established according to the criteria of the new WHO classification, by morphologic and immunophenotypic analysis of paraffin-embedded and fresh-frozen tissue sections, using standard staining methods, as described previously. Of the 63 patients, 54 had single organ (stomach) and 9 had multiple organ involvement (Table 1). Among the 63 patients with gastric MALT lymphomas, we performed genetic analysis, including analysis of the clonality of the CDR3 region and t(11;18)(q21;q21), in 19 patients; 5 with multiorgan involvement, 12 with a single gastric lesion, and 2 with multifocal intragastric lesions (Table 2). *H. pylori* infection was confirmed by positive results of at least three of the following methods of examination; culture, rapid urease test, microscopic examination with Giemsa staining, serum anti-*H. pylori* antibody, and urea breath test. *H. pylori* eradication was performed in 24 patients (21 with gastric involvement alone and 3 with multiorgan involvement).

**Microscopic dissection and DNA extraction**

Microscopic dissection and DNA extraction were performed according to methods described elsewhere. Briefly, 16 serial 10-µm-thick tissue sections were cut in each patient. The first and last sections were stained with hematoxylin and eosin, to assure a high tumor content, and these sections were used to guide the following microdissection. The fresh-frozen tissue sections were visualized under polarized light, and an area with low-grade MALT lymphoma was scraped for API2-MALT1 fusion transcripts and CDR analysis. Genomic DNA derived from separate nontumor tissues served as a control. Paraffin-embedded/formalin-fixed tissue sections, deparaffinized using the DEXPAT kit (Takara, Shiga, Japan), were collected, and DNA extraction was performed, using proteinase K and phenol-chloroform, according to routine molecular biology protocols.

**Reverse transcription-polymerase chain reaction (RT-PCR) for API2-MALT1 fusion transcripts**

API2-MALT1 fusion transcripts were investigated in 15 patients with gastric MALT lymphoma, according to the original method of Inagaki et al reported previously. The fusion transcripts were investigated in 5 of 9 patients with multiorgan involvement, because paraffin sections were not available for 4 patients. Briefly, total RNA was extracted from the paraffin sections by proteinase K digestion. RNA was subjected to first-round multiplex one-tube RT-PCR with three different primer pairs, as described previously, then RNA was subjected to three different second-round nested multiplex PCRs, with each specific primer pair. The final PCR products were stained with ethidium bromide and run on 8% polyacrylamide gels. The band size ranged from 80 to 179 bp. RNA samples known to possess the API2-MALT1 fusion were used as positive controls. As an internal control for RNA quality, the ubiquitously expressed beta-actin mRNA fragment (190 bp) was amplified. In all cases positive for the API2-MALT1

<table>
<thead>
<tr>
<th>Involved organ</th>
<th>No. of patients</th>
<th>H. pylori-positive</th>
<th>Eradication</th>
<th>Remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach alone</td>
<td>54</td>
<td>51 (94.4)</td>
<td>21</td>
<td>15 (71.4)</td>
</tr>
<tr>
<td>Multiple organs</td>
<td>9</td>
<td>3 (33.3)</td>
<td>3</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>54 (85.7)</td>
<td>24</td>
<td>16 (66.7)</td>
</tr>
</tbody>
</table>

*P < 0.05
Values in parentheses are percentages
Eradiation, number of patients who received eradication therapy; remission, number of patients who obtained remission of MALT lymphoma after eradication therapy