Brief report

High incidence of t(11;18)(q21;q21) in Helicobacter pylori–negative gastric MALT lymphoma

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In approximately 5% to 10% of gastric mucosa–associated lymphoid tissue (MALT) lymphomas, evidence of Helicobacter pylori infection is absent, and their pathogenesis is poorly understood. We reviewed the clinical data and histology, and we examined t(11;18)(q21;q21) and BCL10 expression pattern in 17 such cases. In each case, the absence of H pylori was confirmed by negative serology and histology/immunohistochemistry. Five cases with stage IIE or above (5 of 6) than those at stage I E (3 of 10). Two t(11;18)(q21;q21)-positive lymphomas were treated by partial gastrectomy and more than 16 years later showed lymphoma relapse in the gastric stump with dissemination to other mucosal sites, poorly responsive to therapy. BCL10 nuclear expression was observed in 7 of 8 t(11;18)(q21;q21)-positive cases and 4 of 7 t(11;18)(q21;q21)-negative cases, including one case suspicious for a BCL10-involved chromosomal translocation. Our results show that t(11;18)(q21;q21) occurs at a high frequency in H pylori-negative gastric MALT lymphomas. Translocation-positive gastric MALT lymphomas tend to be aggressive, and patients with such lymphomas might benefit from prompt treatment and close follow-up.

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Introduction

Most gastric mucosa-associated lymphoid tissue (MALT) lymphomas are associated with Helicobacter pylori infection. Colonization of the gastric mucosa by the organism induces lymphoid infiltration and formation of acquired MALT, from which the malignant clone arises. The lymphoma growth, particularly at early phases, critically depends on H pylori–specific tumor-infiltrating T cells, involving CD40 and CD40L costimulating molecules. Eradication of H pylori by antibiotics leads to complete regression of gastric MALT lymphoma in 70% of cases and is now used as first-line therapy for this tumor.

Genetically, t(1;14)(p22;q32) and t(11;18)(q21;q21) are implicated in the development of H pylori–associated gastric MALT lymphoma. The t(1;14)(p22;q32) translocation occurs in around 5% of MALT lymphomas and causes deregulation of BCL10 that specifically relays the antigen receptor signaling to nuclear factor κB (NF-κB) pathway. Lymphoma cells with t(1;14)(p22;q32) show strong BCL10 expression in both the nucleus and cytoplasm, in contrast to normal B cells that express the protein only in the cytoplasm and at a much lower level. Nuclear expression of BCL10 at moderate intensity is found in MALT lymphomas without t(1;14)(p22;q32), particularly in those at advanced stages or with t(11;18)(q21;q21).

The t(11;18)(q21;q21) translocation occurs in 30% of gastric MALT lymphomas. The translocation fuses the amino terminal of the API2 gene to the carboxyl terminal of the MALT1 gene and generates a fusion product. The API2-MALT1 fusion product activates NF-κB, a transcriptional factor for a number of survival-related genes. In line with this, gastric MALT lymphoma with t(11;18)(q21;q21) is often at advanced stages and does not respond to H pylori eradication.

In approximately 5% to 10% of gastric MALT lymphomas, there is no evidence of H pylori infection, and their pathogenesis is poorly understood. We reviewed the clinical data and histology of 17 such cases and examined them for t(11;18)(q21;q21) and the pattern of BCL10 expression.

Study design

Patients and materials

Seventeen cases of H pylori–negative gastric MALT lymphoma were retrieved from surgical files of the authors’ institutions. The diagnosis of gastric MALT lymphoma was made according to histologic criteria supported by research grants from the Leukemia Research Fund, United Kingdom.

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described by Isaacson et al. In each case, the absence of *H pylori* was confirmed by serology and histology/immunohistochemistry or by urease test (CLO) and histology/immunohistochemistry. In 4 cases, the absence of *H pylori* was further supported by bacterial culture. Formalin-fixed and paraffin-embedded tumor tissues from diagnostic biopsies were available in each case, and frozen tissues were available in 5 cases. In 6 cases, multiple formalin-fixed and paraffin-embedded tumor tissues were available, as patients underwent partial or total gastrectomy. Histology and clinical data were reviewed.

Reverse-transcription and polymerase chain reaction (RT-PCR) for detection of t(11;18)(q21;q21)

RNA extraction, cDNA synthesis, and PCR amplification were carried out as described previously. In 5 cases in which fresh frozen tissues were available, total RNA was extracted from frozen tissues, and RT was carried out using SuperScript Preamplification System (Invitrogen, Paisley, United Kingdom) with oligo(dT) primer. The API2-MALT1 fusion transcript was amplified by PCR using a pair of primers (f-S and f-AS) that covered all the known breakpoints (Figure 1). PCR products were analyzed on agarose gels.

In the remaining cases, formalin-fixed and paraffin-embedded tissues from diagnostic biopsies were used for RNA extraction. Reverse-transcription was carried out using a mixture of gene-specific primers, including glucose-6-phosphate dehydrogenase (*G6PD*) primers, which were specially designed for formalin-fixed paraffin-embedded tissues. Three sets of PCR primers with one common sense primer covering 93% of the known breakpoints on the *API2* gene and 3 antisense primers targeting all 4 breakpoints on the *MALT1* gene were used for PCR of the *API2-MALT1* fusion transcript (Figure 1). The *G6PD* was amplified in parallel as a control. PCR was performed separately with each primer set at least in duplicate, and the PCR products were analyzed on 10% polyacrylamide gels.

Where indicated, PCR products were gel purified (QIAquick Gel Extraction kit; Qiagen) and sequenced in both directions using dRhodamine dye terminators on an ABI Prism 377 sequencer (PE Applied Biosystems, Foster City, CA).

BCL10 immunohistochemistry

BCL10 was immunostained with a mouse monoclonal antibody (clone 151) on formalin-fixed and paraffin-embedded tissues as described previously.

Results and discussion

The cases used in this study were selected purely based on their absence of *H pylori* infection as confirmed by negative serology and histology/immunohistochemistry or urease test (CLO) and histology/immunohistochemistry. The enzyme-linked immunosorbent assay for detection of serum *H pylori* immunoglobulin G (IgG) antibody is sensitive, capable of detecting 95% of cases. In 7 cases, patient’s sera were also tested for CagA antibodies and none of them were positive. CagA is highly immunogenic, and antibodies to CagA are frequently detected in patient’s sera that are negative for *H pylori* IgG antibody. *H pylori* detection by histologic and immunohistochemical examination is less sensitive and is often affected by the quality and nature of biopsies, but nonetheless it can diagnose around 70% of cases. In the present series, multiple biopsies obtained at various stages of patient’s treatment were available in each case, and all biopsies consistently showed absence of *H pylori* infection. Thus, the cases included in this study were most likely negative for *H pylori* infection.

The cases studied were also unlikely positive for non-*H pylori* helicobacters, such as *Helicobacter heilmannii* and *Helicobacter felis*, which are also associated with human diseases, including gastric MALT lymphoma. Histologic examination of multiple biopsies in each case failed to reveal any direct evidence of presence of bacteria in the gastric mucosa. Gastric MALT lymphomas associated with *H heilmannii* infection have been shown to respond to antibiotic therapy. Of the cases studied, 5 cases with stage I gastric lymphoma were first treated with antibiotics, and none of them showed any endoscopic and histologic response during 4.5 to 12 months of follow-up.

Of the 17 cases studied, clinical staging was available in 16 cases (Table 1), with 10 cases at stage I, and the remaining 6 cases at stage II or above. As mentioned earlier, 5 of the 10 cases with stage I disease were first treated with antibiotics but failed to respond. They were subsequently treated by total gastrectomy or radiotherapy. Of the remaining patients, cases 1 and 2 were first treated by partial gastrectomy because of gastric ulcer, and a review of histology of the gastrectomy specimens showed existence of MALT lymphoma in each case. More than 16 years later, both patients showed occurrence of MALT lymphoma in the gastric stump and in additional mucosal sites (lung in case 1; colon and terminal ileum in case 2). Clonal analysis of the rearranged immunoglobulin heavy chain gene showed that the recurrent tumors were clonally related to the original gastric MALT lymphoma in each case. Both patients were treated with rituximab, with one patient showing only partial response and the other stable disease. All the remaining patients were treated with chemotherapy or partial gastrectomy on diagnosis of MALT lymphoma.
MALT lymphoma has been reported to range from 30% to 50%. The number of cases studied in these reports was relatively small, and the investigation was commonly based on tissue biopsies. As tissue biopsies were small and often distorted, we focused on the 6 cases with gastrectomy specimens, in which multiple formalin-fixed and paraffin-embedded tissues were available. In essence, H pylori-negative gastric MALT lymphomas showed similar histologic features to those associated with H pylori. Characteristic lymphoepithelial lesions, similar extents of reactive lymphoid follicles, and tumor-infiltrating T cells were seen in H pylori–negative gastric MALT lymphomas. These observations suggested that these lymphomas like H pylori–positive cases were most likely arising from acquired MALT. However, the agent that induced the acquired MALT in these cases remains enigmatic.

The frequency of t(11;18)(q21;q21) in H pylori–positive gastric MALT lymphoma has been reported to range from 30% to 50%. The number of cases studied in these reports was relatively small, and the investigation was commonly based on those treated by gastrectomy, which was often biased toward the advanced cases. Thus, these studies may have overestimated the incidence of t(11;18)(q21;q21) positive gastric MALT lymphomas (Table 1). Thus, the incidence of t(11;18)(q21;q21) in H pylori–negative gastric MALT lymphoma is much higher than that of H pylori–positive cases. This finding is in line with the previous finding that the

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Staging</th>
<th>Methods for H pylori detection</th>
<th>Antibiotic therapy</th>
<th>Other treatment and follow-up</th>
<th>t(11;18) breakpoint</th>
<th>BCL10 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>63</td>
<td>IV E</td>
<td>His/serology</td>
<td>ND</td>
<td>Partial gastrectomy because of gastric ulcer (presence of MALT lymphoma on review) in 1982; lymphoma relapsed in the gastric stump and lung in 2001 and treated with rituximab; alive, PR, follow-up for 18 mo</td>
<td>+</td>
<td>1150 Nuc</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>67</td>
<td>IV E</td>
<td>His/serology</td>
<td>ND</td>
<td>Partial gastrectomy because of gastric ulcer (presence of MALT lymphoma on review) in 1985; lymphoma relapsed in gastric stump, colon, and terminal ileum in 2001, treated with rituximab; alive, stable disease, follow-up for 23 mo</td>
<td>+</td>
<td>814 Nuc</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>57</td>
<td>II E</td>
<td>His/serology</td>
<td>ND</td>
<td>Chemotherapy, alive, CR, follow-up for 42 mo</td>
<td>+</td>
<td>1123 Nuc</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>74</td>
<td>I E</td>
<td>His/serology</td>
<td>ND</td>
<td>New case, follow-up not available</td>
<td>+</td>
<td>1123 Nuc</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>65</td>
<td>II E</td>
<td>His/serology/CLO</td>
<td>ND</td>
<td>New case, follow-up not available</td>
<td>+</td>
<td>1123 Nuc</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>38</td>
<td>II E</td>
<td>His/serology</td>
<td>ND</td>
<td>CHOP therapy (9 courses); alive, CR, follow-up for 46 mo</td>
<td>+</td>
<td>814 Cyt</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>55</td>
<td>NA</td>
<td>His/CLO</td>
<td>ND</td>
<td>NA</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>60</td>
<td>I E</td>
<td>His/serology</td>
<td>NR, 4 mo</td>
<td>Total gastrectomy; alive, CR, follow-up for 50 mo</td>
<td>+</td>
<td>814 ND</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>51</td>
<td>I E</td>
<td>His/serology</td>
<td>NR, 4.5 mo</td>
<td>Total gastrectomy because of bleeding, relapsed in lung after 36 mo, salvaged with chemotherapy; alive, CR, follow-up for 70 mo</td>
<td>+</td>
<td>1123 Nuc</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>50</td>
<td>I E</td>
<td>His/serology</td>
<td>NR, 12 mo</td>
<td>Radiotherapy; alive, CR, follow-up for 12 mo</td>
<td>–</td>
<td>NA Cyt</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>45</td>
<td>I E</td>
<td>His/serology</td>
<td>NR, 9.5 mo</td>
<td>Total gastrectomy; alive, CR, follow-up for 42 mo</td>
<td>–</td>
<td>NA Nuc</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>45</td>
<td>I E</td>
<td>His/serology</td>
<td>NR, NA</td>
<td>No other treatment; alive, follow-up for 13 mo</td>
<td>–</td>
<td>NA Cyt</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>53</td>
<td>I E</td>
<td>His/serology</td>
<td>ND</td>
<td>Chemotherapy, lymphoma relapsed and salvaged by radiotherapy; alive, CR, follow-up for 40 mo</td>
<td>–</td>
<td>NA ND</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>64</td>
<td>IV E</td>
<td>His/serology</td>
<td>ND</td>
<td>Concurrent gastric and small intestinal MALT lymphoma, chemotherapy; alive, CR, follow-up for 16 mo</td>
<td>–</td>
<td>NA ND</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>70</td>
<td>I E</td>
<td>His/serology</td>
<td>ND</td>
<td>NA</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>45</td>
<td>I E</td>
<td>His/serology</td>
<td>ND</td>
<td>Partial gastrectomy; new case, follow-up not available</td>
<td>–</td>
<td>NA Nuc</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>57</td>
<td>I E</td>
<td>His/serology</td>
<td>ND</td>
<td>NA</td>
<td></td>
<td>–</td>
</tr>
</tbody>
</table>

His, histology; ND, not done; PR, partial remission; Nuc, nuclear; CR, complete remission; CHOP, a regimen of cyclophosphamide, hydroxydaunomycin, Oncovin (vincristine), and prednisolone; Cyt, cytoplasmic; NA, not available; NR, no response; +, t(11;18) positive; –, t(11;18) negative.

*According to the Ann Arbor system modified by Musshoff.
†According to cDNA sequence of the MALT1 gene (GenBank no. AF130356).
translocation has been found in 2 of 2 H pylori-positive gastric MALT lymphomas.14

Similar to H pylori–positive gastric MALT lymphoma, the occurrence of t(11;18)(q21;q21) in H pylori–negative cases was significantly associated with more advanced cases, being detected in 3 of 10 cases at stage I but in 5 of 6 cases at stage II or above (P < .05, chi-square test). Notably, 2 patients with t(11;18)(q21;q21)-positive gastric MALT lymphoma (nos. 1 and 2) were initially treated by partial gastrectomy, and more than 16 years later both patients showed lymphoma relapse in the gastric stumps and disseminated lesions in additional mucosal sites, which were poorly responsive to therapy with rituximab. A further case (no. 9) underwent total gastrectomy and 36 months later showed lymphoma relapse in lung. The findings highlight 2 important issues. First, it further questions the role of gastrectomy in treatment of gastric MALT lymphoma because the lymphoma cells are widely disseminated in the gastric mucosa and cannot be completely cleared by a partial gastrectomy.77 Second, t(11;18)(q21;q21)-positive cells are capable of surviving and remain dormant for a long period before forming relapse and disseminated lesions, which could impose a challenge for clinical treatment. Thus, it is tempting to speculate that effective systemic treatment administered at an earlier time could be beneficial in such patients.

Of the 15 cases in which immunohistochemistry for BCL10 was performed, one case (no. 17) showed strong BCL10 nuclear expression in most of the tumor cells, similar to that seen in t(1;14)(p22;q32)-positive MALT lymphoma. In the remaining 14 cases, 7 of the 8 t(11;18)(q21;q21)-positive cases and 3 of the 6 translocation-negative cases displayed a BCL10 nuclear expression at a moderate level. The remaining cases exhibited BCL10 expression in the cytoplasm. These findings are similar to those observed in H pylori–positive gastric MALT lymphomas.8

In conclusion, t(11;18)(q21;q21) occurs at a high frequency in H pylori–negative gastric MALT lymphomas. Patients with H pylori–negative gastric MALT lymphoma should be treated with chemotherapy or radiotherapy on diagnosis. The t(11;18)(q21;q21)-positive tumors may require close follow-up.

References