Helicobacter pylori and mucosa-associated lymphoid tissue: what’s new

Sung-Hsin Kuo1,4 and Ann-Lii Cheng1,4

Departments of 1Oncology and 2Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan; and 3Cancer Research Center and 4Graduate Institute of Oncology, National Taiwan University College of Medicine, Taipei, Taiwan

Low-grade mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach, gastric MALT lymphoma, is associated with Helicobacter pylori infection. The eradication of H. pylori using antibiotics is successful in 60% to 80% of affected patients. In contrast to the previous paradigm, we and other investigators have shown that a certain proportion of patients with H. pylori–positive early-stage diffuse large B-cell lymphoma (DLBCL) of the stomach with histological evidence of MALT lymphoma, including high-grade transformed gastric MALT lymphoma and gastric DLBCL(MALT), achieved long-term complete pathological remission (pCR) after first-line H. pylori eradication therapy, indicating that the loss of H. pylori dependence and high-grade transformation are separate events in the progression of gastric lymphoma. In addition, patients with H. pylori–positive gastric DLBCL without histological evidence of MALT (gastric pure DLBCL) may also respond to H. pylori eradication therapy. A long-term follow-up study showed that patients who achieved pCR remained lymphoma free. Gastric MALT lymphoma is indirectly influenced by H. pylori infection through T-cell stimulation, and recent studies have shown that H. pylori–triggering chemokines and their receptors, H. pylori–associated epigenetic changes, H. pylori–regulated miRNA expression, and tumor infiltration by CD4+ CD25+ regulatory T cells contribute to lymphomagenesis of gastric MALT lymphoma. Recent studies have also demonstrated that the translocation of CagA into B lymphocytes inhibits apoptosis through p53 accumulation, BAD phosphorylation, and the up-regulation of Bcl-2 and Bcl-XL expression. In gastric MALT lymphoma, CagA may stimulate lymphomagenesis directly, through the regulation of signal transduction, and intracellular CagA is associated with H. pylori dependence. These findings represent a substantial paradigm shift compared with the classical theory of H. pylori–reactive T cells contributing indirectly to the development of MALT lymphoma. In conclusion, a wide range of H. pylori–related gastric lymphomas have been identified. The use of antibiotics as the sole first-line therapy for early-stage gastric pure DLBCL requires validation in a prospective study. The clinical and biological significance of the CagA oncoprotein in the lymphomagenesis of gastric MALT lymphoma warrants further study.

Introduction

With a relatively high incidence and unique clinicopathological features, gastric lymphoma has recently become an important area of cancer research. In 1983, Isaacson and Wright described a group of extranodal low-grade B-cell lymphomas derived from Peyer patch–like structures of mucosa-associated lymphoid tissue (MALT) in the stomach, salivary glands, lungs, and thyroid gland.1 These types of tumors have since been recognized as a discrete group of indolent B-cell-lineage lymphomas and are classified as extranodal marginal zone lymphomas of the MALT type in the Revised European-American Lymphoma classification system of the World Health Organization (WHO).2 Since 2008, the term “low-grade MALT lymphoma” has not been used in the WHO classification system because MALT lymphoma is low grade by definition.3

Recognizing MALT lymphoma as a distinct lymphoma entity, Marshall and Warren described direct links to Helicobacter pylori infection, gastritis, and peptic ulcer disease.4 Gastric low-grade MALT lymphoma (gastric MALT lymphoma) consists primarily of diffuse small- and medium-sized centrocyte-like cells and lymphoepithelial lesions.5 In the early 1990s, Wotherspoon et al first described a high incidence of H. pylori–related gastritis among gastric MALT lymphoma patients and demonstrated that lymphoid follicles morphologically similar to healthy MALT developed in H. pylori–infected patients.5 Because the stomach is normally devoid of organized lymphoid tissues, this finding suggests that the acquisition of gastric MALT in H. pylori infection facilitates the development of gastric MALT lymphoma.6

Wotherspoon et al demonstrated that H. pylori eradication therapy (HPE) alone resulted in the histological regression of gastric MALT lymphoma in 5 of 6 cases (83.3%).7 Epidemiological and case-control studies have revealed a strong positive correlation between H. pylori and gastric MALT lymphoma and independent clinical studies have demonstrated that complete pathological remission (pCR) in 56% to 100% of patients with H. pylori–positive early-stage gastric MALT lymphoma can be achieved through HPE.8 In addition, in vitro studies showed that the growth of MALT lymphomas requires the assistance of H. pylori–reactive tumor-infiltrating T cells,8,9 and the development of gastric lesions histopathologically resembling features of human gastric MALT lymphoma was demonstrated in a BALB/c mouse model of H. pylori infection.10

A recent systematic review of 32 reports of gastric MALT lymphoma in 1408 patients showed that 77.5% achieved pCR after
successful HPE\textsuperscript{11} and remission was achieved within 12 months of HPE in most cases.\textsuperscript{11,12} However, these studies evaluated post-HPE tumor regression using different histological grading systems. In some studies, tumors that had resolved to Wotherspoon grade 2 (chronic active gastritis with florid lymphoid follicle formation) or less were defined as pCR.\textsuperscript{7} In contrast, Neubauer et al defined pCR as tumors with no remaining lymphoma cells and an “empty” tunica propria with small basal clusters of lymphocytes and scattered plasma cells.\textsuperscript{13}

The histological scoring system proposed by Groupe d’Etude des Lymphomes de l’Adult (GELA) is currently recommended to improve the consistency between the findings of different studies.\textsuperscript{12} The pCR is defined by the GELA grading system as the total disappearance of lymphoid infiltrate with scattered small lymphocytes and plasma cells and regressive stromal changes with fibrosis and separation of the glands. Complete clinical remission is defined as no macroscopic findings of lymphoma and negative histological findings equivalent to pCR or the presence of small lymphoid aggregates at the base of the lamina propria representing probable minimal residual disease in at least 2 subsequent follow-up examinations.

Delayed pCR has been reported in some gastric MALT lymphoma patients,\textsuperscript{11} whereas reports of relapse after pCR are extremely rare. Zulio et al reported a relapse rate of 7.2% in 994 patients during a follow-up evaluation of 3253 patient-years.\textsuperscript{11} In gastric MALT lymphoma patients achieving pCR through HPE, reinfection with the same strain of \textit{H pylori} has been shown to increase recurrence.\textsuperscript{11,12} Therefore, careful follow-up screening for \textit{H pylori} reinfection is strongly recommended.

In contrast to gastric MALT lymphoma, gastric diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease that has been traditionally treated with systemic chemotherapy.\textsuperscript{2,5,14} Gastric DLBCLs are manifested as compact clusters, confluent aggregates, or sheets of large lymphoma cells with distinctive nucleoli.\textsuperscript{2,5,14} Gastric DLBCLs are classified as those with or without features of MALT lymphoma according to the current WHO lymphoma classification criteria.\textsuperscript{2,3,14} High-grade transformed MALT lymphoma of stomach, gastric DLBCL(MALT), is DLBCL with features of MALT lymphoma and should not be classified strictly as MALT lymphoma. Treatment of gastric DLBCL(MALT) may not respond to HPE. However, studies have reported that a substantial proportion of \textit{H pylori}–positive early-stage gastric DLBCL(MALT) patients achieved remission through HPE.\textsuperscript{15,20}

Our previous study revealed that gastric DLBCL without histological evidence of MALT (gastric pure DLBCL) can also be cured by HPE,\textsuperscript{21} which represents a revolutionary finding in the treatment of gastric lymphoma. Most importantly, the spectrum of \textit{H pylori}–related lymphomas is much wider than previously thought. This review describes the evolution of our understanding of \textit{H pylori}–related gastric lymphoma. We discuss new insights into the molecular mechanisms of \textit{H pylori}–induced gastric MALT lymphoma focusing on indirect \textit{H pylori} antigen-driven lymphomagenesis and direct interactions between \textit{H pylori} and lymphoma B cells.

**High-grade transformation does not confer \textit{H pylori} independence of gastric MALT lymphoma**

Similar to gastric MALT lymphoma patients, prospective studies and 1 retrospective study reported that some early-stage \textit{H pylori}–positive gastric DLBCL(MALT) patients can also achieve long-term pCR after first-line HPE (Table 1).\textsuperscript{15-21} Most of these patients were treated with antibiotics for 2 weeks and had multiple sequential follow-up endoscopic examinations to monitor disease progression or to verify pCR. Successful eradication of \textit{H pylori} was achieved in 96.5% (56/58) of patients, resulting in a pCR rate of 58.9% (33/56). The median time to pCR after completion of HPE was 4.0 months (95% confidence interval: 2.1-5.9) and all patients who achieved pCR remained lymphoma free after a long-term follow-up.

In our previous study of the relationship between the depth of tumor infiltration and the tumor response, the pCR rate of tumors limited to the mucosa or submucosa and that of tumors in the muscularis propria or beyond was 80% (8/10) and 29.4% (5/17), respectively (\(P = .018\)), whereas a similar Japanese study reported 66.7% (4/6) and 25% (1/4), respectively (\(P = .524\)).\textsuperscript{17,21} A recent analysis of 1215 MALT lymphoma and 56 DLBCL(MALT) patients showed that the tumor regression rate after successful HPE was higher in MALT lymphoma patients than in DLBCL(MALT) patients (78.5% vs 62%; \(P = .0062\)).\textsuperscript{22} Although these data suggest that HPE may be considered a treatment option for early-stage \textit{H pylori}–positive gastric DLBCL(MALT), large-scale prospective studies are required to validate its use as a first-line therapy.

**\textit{H pylori}–positive gastric pure DLBCL is dependent on \textit{H pylori} and can be cured by HPE**

In contrast to DLBCL(MALT), gastric DLBCL without histologic evidence of MALT (gastric pure DLBCL) is generally thought to originate de novo rather than from the transformation of MALT lymphoma and is therefore considered unrelated to \textit{H pylori} infection. However, epidemiological and case-control studies have demonstrated an association between \textit{H pylori} infection and gastric DLBCL.\textsuperscript{2,3,24} with prevalence rates of \textit{H pylori} infection in gastric DLBCL patients of 85% to 89%.\textsuperscript{23,24}

Similar to recent anecdotal case reports showing that HPE can achieve pCR in early-stage gastric pure DLBCL patients,\textsuperscript{25} our recent exploratory study demonstrated a high level of efficacy for first-line antibiotic therapy in early-stage (stage IE/IIIE1) \textit{H pylori}–positive gastric pure DLBCL patients (Table 1).\textsuperscript{21} In our study, patients received their first upper-gastrointestinal endoscopic follow-up examination between 4 and 6 weeks after completing antibiotic therapy.\textsuperscript{21} The median interval from HPE to pCR was 2.1 months. Eleven patients achieved pCR after HPE and were free of lymphoma at a median follow-up of 3.9 years, except one patient who died of lung cancer.\textsuperscript{21}

Our results are consistent with those of a recent multicenter phase 2 study showing an efficacy rate of 50% for first-line antibiotic treatment for high-grade gastric lymphoma in 5 DLBCL(MALT) and 11 pure DLBCL patients (Table 1).\textsuperscript{26} In a Japanese retrospective study, Tari et al reported that 4 (26.7%) of 15 patients with stage IE \textit{H pylori}–positive gastric pure DLBCL achieved pCR after HPE, with a median interval to pCR of 3 months (Table 1), and all patients who achieved pCR had tumor invasion of the mucosa or shallow lesions of the submucosa.\textsuperscript{27}

Our study also showed that 3 (42.9%) of 7 patients with gastric pure DLBCL tumor extensions to perigastric lymph nodes (> 1 cm, stage IIE1) achieved pCR after HPE, suggesting that tumor extension my affect the tumor response to HPE.\textsuperscript{21} Ferreri et al also showed that the presence of small perigastric lymph nodes (< 1.5 cm) around a tumor is not a contraindication for first-line antibiotic
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>N, M/F</th>
<th>Age, y</th>
<th>Histology</th>
<th>Stage</th>
<th>HPE regimen (for 2 wk)</th>
<th>pCR rate</th>
<th>Median time to pCR, mo</th>
<th>Follow-up period for pCR</th>
<th>Salvage treatment for non-pCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morgner et al</td>
<td>Germany</td>
<td>4/4</td>
<td>26-85</td>
<td>DLBCL(MALT)</td>
<td>IE: 6</td>
<td>Dual regimen (2 wk): omeprazole 40 mg TID, amoxicillin 750 mg TID</td>
<td>7/8 (87.5%)</td>
<td>1-4</td>
<td>6-66 mo</td>
<td>CHOP</td>
</tr>
<tr>
<td>Nakamura et al</td>
<td>Japan</td>
<td>10/NA</td>
<td>NA</td>
<td>DLBCL(MALT)</td>
<td>IE</td>
<td>Proton pump inhibitor plus clarithromycin 600 mg/d, amoxicillin 2000 mg/d, metronidazole 800 mg/d</td>
<td>5/10 (50%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hiyama et al</td>
<td>Japan</td>
<td>4/NA</td>
<td>NA</td>
<td>DLBCL(MALT)</td>
<td>IE</td>
<td>Proton pump inhibitor plus antibiotics for 1-2 wk</td>
<td>2/4 (50%)</td>
<td>6</td>
<td>18 mo</td>
<td>CHOP</td>
</tr>
<tr>
<td>Alpen et al</td>
<td>Germany</td>
<td>2/NA</td>
<td>73-76</td>
<td>DLBCL(MALT)</td>
<td>IE</td>
<td>Omeprazole 40 mg/d, clarithromycin 500 mg BID, metronidazole 20 mg BID</td>
<td>1/2 (50%)</td>
<td>1.3</td>
<td>5.7 mo</td>
<td>CHOP</td>
</tr>
<tr>
<td>Chen et al</td>
<td>Taiwan</td>
<td>12/22</td>
<td>55 (35-83)</td>
<td>DLBCL(MALT)</td>
<td>IE: 30</td>
<td>Amoxicillin 500 mg 4 times/d</td>
<td>18/32 (56.3%)</td>
<td>5.0 (95% CI, 2.8-7.5)</td>
<td>11.7 y (95% CI, 7.8-14.4)</td>
<td>CHOP, CEOP,</td>
</tr>
<tr>
<td>Kuo et al</td>
<td>Taiwan</td>
<td>6/10</td>
<td>34-88 (median 63)</td>
<td>PureDLBCL</td>
<td>IE: 1</td>
<td>Clarithromycin 500 mg 4 times/d, omeprazole 20 mg BID</td>
<td>11/16 (68.8%)</td>
<td>2.1 (95% CI, 0.7-6.3)</td>
<td>3.5 y (95% CI, 0.7 - 6.3)</td>
<td>R-CHOP or R-CEOP</td>
</tr>
<tr>
<td>Ferreri et al</td>
<td>Italy</td>
<td>16/NA</td>
<td>NA</td>
<td>Pure: 11 DLBCL (MALT): 5</td>
<td>IE</td>
<td>Clarithromycin 500 mg BID, tinidazole or metronidazole 500 mg BID, omeprazole 20 mg BID</td>
<td>8/16 (50%)</td>
<td>NA</td>
<td>1.4-114 mo; 9/10 patients CR remain lymphoma free</td>
<td>PR: rituximab; SD or PD: chemo-RT</td>
</tr>
<tr>
<td>Tari et al</td>
<td>Japan</td>
<td>7/8</td>
<td>63-91 (mean 72.9)</td>
<td>PureDLBCL</td>
<td>IE</td>
<td>Rabeprazole 20 mg/d, amoxicillin 750 mg BID, metronidazole 200 mg BID</td>
<td>4/15 (26.7%)</td>
<td>3</td>
<td>7.100 mo (no recurrence)</td>
<td>R-CHOP-21 followed by RT</td>
</tr>
</tbody>
</table>

NA indicates not analyzed; R, rituximab; BID, twice daily; CI, confidence interval; TID, 3 times daily; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone; RT, radiotherapy; CEOP, cyclophosphamide, epirubicin, vincristine, and prednisolone; PR, partial remission; SD, stable disease; and PD, progressive disease.

*Perigastric lymph nodes of diameter < 1.5 cm.
Previous studies have shown that HPE combined with chemotherapy, or radiotherapy (Table 1).21,26,27 Overall, these findings indicate that a substantial number of H pylori–positive gastric pure DLBCLs remain sensitive to HPE. 21,26,27,29 Clarifying the significance of H pylori infection in the development of gastric pure DLBCL is therefore important.

Previous studies have found that the genetic aberrations and molecular features in the large-cell components of gastric DLBCL (MALT) overlap with those of gastric DLBCL, suggesting that a certain portion of gastric pure DLBCL may be blastic variants of marginal zone lymphoma.30,31 Patients with gastric pure DLBCL have a more favorable clinical course compared with those with nodal DLBCL.32 Previous studies have shown that HPE combined with chemotherapy in gastric pure DLBCL patients can increase treatment efficacy compared with chemotherapy alone.33 We also found that H pylori–positive gastric pure DLBCL patients displayed a lower rate of tumor progression, better tumor response to chemotherapy, and better overall survival than those without H pylori infection.34 These findings indicate that H pylori–related gastric pure DLBCL may share common clinicopathological and biological features with H pylori–related gastric MALT lymphoma.

### Lymphomagenesis of H pylori–related gastric MALT lymphoma (Table 2)

**Indirect effects of H pylori antigens and infiltrating H pylori–specific T cells in gastric MALT lymphoma tumorigenesis**

In the early process of MALT lymphomagenesis, the proliferation response is partially dependent on the stimulation of H pylori–specific intratumoral T cells by H pylori antigens.8,9,35 In vitro experiments have demonstrated that the growth and differentiation of MALT lymphoma cells require CD40-mediated signaling and T helper-2 (Th-2)–type cytokines.9,35 In addition to promoting the proliferation and differentiation of malignant B-cell clones, tumor-infiltrating T cells in gastric MALT lymphomas have been found to be defective in both perforin-mediated cytotoxicity and Fas-Fas ligand-mediated apoptosis.9,35

For effective communication between T cells and neoplastic B cells, interactions between 2 subsets of costimulatory molecules of neoplastic B cells, CD80 (B7.1) and CD86 (B7.2), and the CD28 or CTLA-4 of T cells is required.35 In a study of gastric MALT lymphoma, CD86 expression was found to be significantly associated with sensitivity to HPE (H pylori dependence), whereas CD80 and CD40 and their ligands were not.36 These findings are consistent with our report of a significant association between CD86 expression in DLBCL(MALT) cells and H pylori dependence.37 In addition to the stimulatory effects of infiltrating T cells on lymphomagenesis, the growth of MALT lymphoma tumors is also triggered by self-antigen–stimulating BCR signaling.38,39 The findings of these previous studies suggest that the dependence on T cells for the growth of malignant B-cell clones may explain the tendency for gastric MALT lymphomas to remain localized and the HPE sensitivity of a substantial proportion of MALT lymphomas and DLBCL(MALT)s of the stomach.

### Possible roles of chemokine receptor-mediated signaling in gastric MALT lymphoma tumorigenesis

In addition to the indirect role of T cells in the lymphomagenesis of gastric MALT lymphoma, a previous study showed that both BCA-1 (a chemokine) and its chemokine receptor CXCR5 are highly expressed in H pylori–associated primary and secondary lymphoid follicles and H pylori–positive gastric MALT lymphoma cells.40 During H pylori infection, BCA-1 constitutively regulates the homing of B-cell lymphocytes to MALT lymphoma cells through BCA-1 receptor-mediated signaling and it also promotes the production of inflammatory cytokines such as IL-8.40 However, studies have shown that CXCR3 expression in MALT lymphoma cells is correlated with the metastatic migration of neoplastic B cells into the circulation and other lymphoid organs, suggesting that this chemokine may be linked with the loss of H pylori dependence and the progression to advanced-stage MALT lymphoma.41 Yamamoto et al also showed that CXCR3 expression was significantly higher in tumors that did not respond to HPE (H pylori independence) than that in H pylori–dependent tumors.42 Furthermore, Deutsch et al demonstrated that the expression of CCR7, CXCR3, and CXCR7 were up-regulated after the malignant transformation of gastric

---

**Table 2. Potential molecular and biologic markers that predict the H pylori–dependent status of early-stage gastric MALT lymphoma patients with first-line antibiotic treatment**

<table>
<thead>
<tr>
<th>Response to HP eradication</th>
<th>Markers</th>
<th>Methods</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP dependence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costimulatory molecules</td>
<td>CD86 (B7.2)</td>
<td>IHC</td>
<td>36,37</td>
</tr>
<tr>
<td>CD4+CD56+ Treg</td>
<td>FOXP3</td>
<td>IHC</td>
<td>47,48</td>
</tr>
<tr>
<td>Methylation</td>
<td>p16INK4A</td>
<td>Methylation-specific PCR</td>
<td>56,58</td>
</tr>
<tr>
<td>HP-specific protein</td>
<td>CagA protein</td>
<td>IHC</td>
<td>50</td>
</tr>
<tr>
<td>HP-specific protein</td>
<td>Serum CagA IgG antibody</td>
<td>ELISA (CagA kit)</td>
<td>68</td>
</tr>
<tr>
<td>HP independence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome</td>
<td>t(11;18)(p21;q21)</td>
<td>RT-PCR or FISH</td>
<td>8,38</td>
</tr>
<tr>
<td>Chromosome</td>
<td>t(1;14)(p22;q32)</td>
<td>FISH</td>
<td>8,38</td>
</tr>
<tr>
<td>Protein</td>
<td>BCL10 nuclear expression</td>
<td>IHC</td>
<td>38,69</td>
</tr>
<tr>
<td>Chemokine/chemokine receptor</td>
<td>CXCR3</td>
<td>IHC</td>
<td>42</td>
</tr>
<tr>
<td>Methylation</td>
<td>MAD2</td>
<td>Methylation-specific PCR</td>
<td>58</td>
</tr>
<tr>
<td>mRNA</td>
<td>E2A</td>
<td>miRNAs RT-PCR</td>
<td>61</td>
</tr>
<tr>
<td>mRNA</td>
<td>miR-203</td>
<td>miRNAs RT-PCR</td>
<td>62</td>
</tr>
<tr>
<td>mRNA</td>
<td>-142-5p and miR-155</td>
<td>miRNAs RT-PCR</td>
<td>63</td>
</tr>
</tbody>
</table>

IHC indicates immunohistochemistry.
MALT lymphoma from *H pylori*–associated gastritis, whereas CXCR4 expression was down-regulated.\(^{43}\)

**CD4\(^+\) CD25\(^+\) regulatory T-cell involvement in gastric MALT lymphoma tumorigenesis**

In the microenvironments of the stomach, *H pylori* infection stimulates the response of CD4\(^+\)CD25\(^+\) regulatory T-cell (Tregs).\(^{44}\) Tumor-infiltrating Tregs in B-cell lymphoma are memory T cells that often express intracellular FOXP3, a master regulatory gene for the development and function of natural Tregs.\(^{45}\) In a BALB/c mouse model of *H felis*–induced MALT lymphoma, lymphoma B-cell proliferation was found to be dependent on both BCR signals and intratumoral CD4\(^+\) T cells.\(^{46}\) Most of the tumor-infiltrating T cells were shown to be Tregs, and these Tregs were recruited by tumor cells through the secretion of the T-reg-attracting chemokines CCL17 and CCL22.\(^{46}\)

Two recent in vivo studies examined the possible mechanisms of Treg involvement in the immunomodulation of gastric MALT lymphoma and evaluated Treg-mediated influences on treatment response.\(^{57,58}\) Garcia et al showed that *H pylori*–dependent tumors contained more FOXP3\(^+\) cells and a higher FOXP3\(^+\)/CD3\(^+\) cell ratio than *H pylori*–independent tumors.\(^{47}\) In addition, Iwaya et al showed that *H pylori*–dependent tumors had more FOXP3\(^+\) cells and higher FOXP3\(^+\)/CD4\(^+\) cell ratios than *H pylori*–independent tumors.\(^{48}\) These findings suggest that a higher number of tumor-infiltrating FOXP3\(^+\) Tregs at baseline is closely associated with *H pylori* dependence.

**Direct effects of *H pylori* on B-cell proliferation in gastric MALT lymphoma**

We recently reported that the *H pylori* protein CagA is translocated into human B lymphocytes. Intracellular CagA coimmunoprecipitated with SHP-2, suggesting that it stimulates the proliferation of B cells by regulating intracellular signaling pathways, such as the activation of ERK and p38 MAP kinase and the up-regulation of expression of Bcl-2 and Bcl-X\(_L\).\(^{49}\) The intracellular CagA appears to act as a bacterium-derived oncprotein that contributes to the pathogenesis of gastric MALT lymphoma (Figure 1).\(^{49}\) We also detected CagA in 69.8% (30/43) of the *H pylori*–dependent cases, whereas CagA was detected in only 14.7% (5/34) of *H pylori*–independent lymphomas (\(P < .001\)).\(^{50}\) In addition, we determined that CagA translocation is highly associated with the expression of CagA signaling pathway–related proteins, such as phospho-SHP-2, phospho-ERK, phospho-p38MAPK, Bcl-2, and Bcl-xl.\(^{51}\) These findings are consistent with those of Ohnishi et al showing that CagA might play a role in the development of *H pylori*–associated gastrointestinal and hematopoietic neoplasms through CagA–mediated tyrosine phosphorylation and subsequent deregulation of SHP-2, based on their studies in a transgenic mouse model.\(^{52}\) Two in vitro studies have also shown that B-lymphocyte apoptosis can be inhibited in the presence of intracellular CagA through the decreased transcription and the accumulation of p53 or the ERK1/2–mediated phosphorylation at Ser\(^{112}\) of BAD.\(^{53,54}\)

Although our findings and those of others suggest that CagA can affect lymphomagenesis directly through the regulation of CagA–related signal transduction in gastric MALT lymphoma, other *H pylori*–related pathways associated with lymphoid neoplasms may also be involved. Our preliminary results showed that polymorphism of IL-22 plays an important role in the development of MALT lymphoma and that IL-22 expression is associated with *H pylori* dependence in gastric MALT lymphoma (F. Liao, Y. C. Hsu, S.H.K., et al, unpublished data, May 15, 2013). In addition to regulating the immune response of Tregs, *H pylori* infection also modulates the expression of Th17 helper cell–related cytokines.\(^{55}\) Based on these findings, we propose that the proliferation of *H pylori*–related gastric lymphoma cells requires at least 3 signals for proliferation, as summarized in Figure 2.

**H pylori–regulated epigenetic changes and miRNA expression are associated with gastric MALT lymphoma tumorigenesis**

Because most *H pylori*–positive gastric MALT lymphomas are sensitive to HPE, epigenetics and other regulatory processes, rather than genetics, may drive the progression of the early stages of MALT lymphoma. Studies have reported that the methylation of the cyclin-dependent kinase inhibitor p16\(^{NK4A}\) is involved in the progression of *H pylori*–associated gastric MALT lymphoma.\(^{56-58}\) Park et al demonstrated that p16\(^{NK4A}\) methylation occurred more frequently in t(11;18)(q21;q21)–negative gastric MALT lymphoma, whereas methylation of the MAD2 gene was associated with BCL10 expression.\(^{58}\) In a recent study on the relationship between

---

**Figure 1. The *H pylori* protein CagA might function as a bacterium-derived oncprotein in the carcinogenesis of gastric MALT lymphoma.**

(A) Translocation of CagA (EGFP) into BJAB (Epstein-Barr virus-negative Burkitt lymphoma) cells is revealed by immunohistochemical staining of cell surface IgM (red) and confocal microscopy. (B) *H pylori*–derived CagA can be translocated into B lymphocytes. Intracellular CagA coimmunoprecipitates with SHP-2, suggesting the involvement of CagA in the regulation of intracellular signaling pathways. Modified with permission from Lin et al.\(^{49}\)
lymphomagenesis of gastric MALT lymphoma. H. pylori infection stimulates T lymphocytes in the gastric mucosa and indirectly induces the formation of MALT, from which B lymphocytes migrate and infiltrate the site of H. pylori infection in the stomach. (A) The CagA protein may be translocated into B lymphocytes as it is secreted by H. pylori on gastric epithelial surfaces, thereby resulting in a cascade of survival signaling in the B lymphocytes. (B) H. pylori infection can also indirectly promote lymphomagenesis through T-cell-stimulatory pathways such as CD40-mediated signaling and the expression of Th-2-type cytokines and costimulatory molecules such as CD86. H. pylori infection can stimulate CD4+CD25+ Tregs expressing FOXP3. (C) Molecular cross-talk between lymphoma B cells and factors in the tumor microenvironment (T cells and Th17 helper cell-regulated cytokines including IL-22, chemokines, and chemokine receptors) stimulate lymphoma cell survival.

the CpG-island-methylator phenotype (CIMP) and gastric lymphoma, Kondo et al showed that H. pylori-positive gastric lymphoma was associated with a significantly higher incidence of CIMP (93%, 27/29) than that of H. pylori-negative gastric lymphoma (42%, 5/12; P < .001).59 Aberrant CpG methylation of certain genes, such as p16, MGMT, and MINT31, was associated with H. pylori infection.59 Therefore, hypermethylation of certain genes may be an important epigenetic mechanism in the development of H. pylori-related gastric lymphoma.

Recent studies have demonstrated that small noncoding miRNAs provide more information than mRNAs for tumorigenic events in H. pylori-induced human cancers, including gastric MALT lymphoma.60-63 Our study showed that the expression of E2A, a transcription factor that induces somatic hypermutation and blocks plasma cell differentiation in MALT lymphoma, was associated with significantly higher levels of miRNA-223 expression in gastric MALT lymphoma cells, which resulted in a poor response to HPE.64 In addition, microarray-based profiling of miRNA expression showed that the expression of miR-203 and its target ABL1 was dysregulated in MALT lymphoma biopsy samples.65 In another recent study, miRNA microarray analysis of tissue specimens from gastric MALT lymphomas and the surrounding nontumor mucosae revealed that miR-142 and miR-155 were expressed at much higher levels in the MALT lymphoma lesions and that the expression levels of miR-142-5p and miR-155 were significantly higher in H. pylori-independent tumors than in H. pylori-dependent tumors.65

New treatment strategies for localized gastric MALT lymphoma that does not respond to HPE

Previous studies have shown that conventional therapeutic modalities such as chemotherapy using chlorambucil, fludarabine, or cyclophosphamide; radiotherapy; and immunotherapy (anti-CD20 antibodies, rituximab) demonstrate various degrees of effectiveness for the treatment of localized gastric MALT lymphomas.12,28,64 However, a substantial proportion of patients with H. pylori–independent gastric lymphoma also fail to respond to chemotherapy or anti-CD20 immunotherapy. Recent studies have reported that BCR signaling inhibitors such as ibrutinib (a Bruton tyrosine kinase inhibitor) and immunomodulatory drugs such as lenalidomide (a derivative of thalidomide) have achieved encouraging clinical outcomes in the treatment of a variety of B-cell lymphomas, including multiple myeloma.65,66 Because of the relationship between multiple myeloma and MALT lymphoma and the role of immunological pathways in lymphomagenesis, further clinical studies of the use of ibrutinib or lenalidomide for the treatment of H. pylori–independent gastric MALT lymphoma are warranted.67

Summary and future outlook

Over the previous 10 years, a wide spectrum of various types of gastric lymphoma have been shown to respond to HPE. In the future, validation of the efficacy of HPE for gastric pure DLBCL is most crucial because it is a relatively common lymphoma that is universally treated with intensive systemic chemotherapy. We have also witnessed a paradigm shift in the study of carcinogenesis in H. pylori–related gastric lymphomas. In addition to the classical “H. pylori → T-cell → marginal-zone B cell” pathway of lymphoma carcinogenesis, H. pylori may also have direct effects on B cells that also contribute to the development of lymphoma. It is equally important to explore the cellular origins of H. pylori–related lymphomas. In addition to the classical “marginal-zone B cell → MALT lymphoma → DLBCL(MALT) → pure DLBCL” pathway of carcinogenesis, the possibility that H. pylori may contribute to the development of lymphoma by transforming B cells other than those in the marginal zone warrants further study.
Acknowledgments
The authors thank Dr Li-Tzong Chen (National Institute of Cancer Research, National Health Research Institutes, Tainan, Taiwan; the Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; and the Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan) for his contributions to our manuscript, for providing detailed information regarding his unpublished data, and for helpful discussions.

Disclosures
Conflict-of-interest disclosure: The authors declare no competing financial interests. Off-label drug use: None disclosed.

Correspondence
Ann-Li Cheng, MD, PhD, Department of Internal Medicine and Department of Oncology, National Taiwan University Hospital, No. 7, Chung-Shan South Rd, Taipei, Taiwan; Phone: 886-2-2312-3456, ext. 67251; Fax: 886-2-2371-1174; e-mail: alcheng@ntu.edu.tw.

References
29. Möller P, Viardot A. Antibiotics as first-line therapy for
62. Craig VJ, Cogliatti SB, Rehrauer H, Wündisch T, Müller A. Epigenetic silencing of microRNA-203 dysregulates ABL1


