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Helicobacter and Gastric Malignancies

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Abstract

Individuals infected with *Helicobacter pylori*, a stomach colonizing bacteria, have an increased risk of developing gastric malignancies. The risk for developing cancer relates to the physiologic and histologic changes that *H. pylori* infection induces in the stomach. In the last year numerous studies have been conducted in order to characterize the association between *H. pylori* infection and gastric cancer. These studies range from epidemiologic approaches aiming at the identification of environmental, host genetic, and bacterial factors associated with risk of gastric cancer, to molecular and cell biology approaches aiming at understanding the interaction between *H. pylori* and the transforming epithelial cell. In this review an account of the last year's research activity on the relationship between *H. pylori* and gastric cancer will be given.

Keywords

Helicobacter pylori; gastric cancer; MALT lymphoma

Gastric Carcinoma

Gastric carcinoma (GC) is one of the most common forms of cancer, with approximately 900,000 new cases diagnosed every year and a leading cause of cancer-related deaths in many parts of the world [1]. Significant advances toward the understanding of gastric carcinogenesis have been achieved since the description of *Helicobacter pylori* by Marshall and Warren, in 1984 [2], and its later classification as a class I carcinogen (by the International Agency for Research on Cancer) [3]. It is now well established that *H. pylori* infection is the major risk factor for the development of noncardia GC. Although almost half of the world's population is infected with the bacteria, colonization is usually asymptomatic

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Conflicts of interest

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and tumor progression only occurs in a subset of individuals and is dependent on the host response as well as genetic variation of the bacteria.

H. pylori Virulence Factors

A major determinant of *H. pylori* virulence is VacA (vacuolating cytotoxin), a cytotoxin that induces cytoplasmic vacuolation in gastric cells [4]. All *H. pylori* strains possess the *vacA* gene, but not all of them induce vacuolation, pointing to the existence of genetic variability within *vac*A. Two major polymorphic regions have been identified: the signal region (that may be type *s1* or *s2*) and the midregion (type *m1* or *m2*) [5]. Malignancy is associated with *s1*/*m1* strains, although some *s1*/*m2* strains have been isolated from GC cases [6]. Current knowledge on this subject was boosted by the work of Rhead et al. [7], who identified a new *vacA* polymorphic site, designated as the intermediate (*i*) region, and two sequence types (*i1* and *i2*). They showed that *s1*/*m1* strains were exclusively *i1* and *s2*/*m2* were exclusively *i2*. Moreover, *i1* strains included all *s1*/*m1* and all *s1*/*m2* strains with vacuolating activity. Significantly, this study demonstrated an association of *i1* with GC in 73 patients from Iran. Furthermore, after a logistic regression analysis of *vacA*, only the *i* region was determined to be an independent marker of GC. These results, although needing further confirmation in other populations, suggest that *i* region typing may be sufficient for the identification of all pathogenic forms of *vacA* and may be very useful for cancer prevention.

The first Asian-Pacific Gastric Carcinoma Consensus reviewed available data concerning *H. pylori* and GC development and recommended that population-based screening and *H. pylori* treatment should be applied, in particular in high-risk populations [8]. As *H. pylori* removal from large sections of the population may be difficult to implement economically and its long-term consequences are still unpredictable, identification of at-risk individuals is thus very important. Whether different risks are more dependent on the bacterial strain variability (or even evolution through time) colonizing the stomach or on the host genetic variability is under investigation.

Kawazoe et al. showed that infection with *H. pylori* strains SS1 (CagA−, VacA−) and TN2GF4 (CagA+, VacA+) promoted carcinogenesis in Mongolian gerbils [9]. Interestingly, results obtained with the SS1 strain contrasted with a previous report from the same authors, where infection of C57BL/6 mice even reduced the risk for GC development. The authors speculate that, although bacterial virulent factors have a role in carcinogenesis, *H. pylori* infection *per se* is more important than the diversity of the infecting strain and that chronic inflammation, irrespective of the degree, and the consequent destruction and proliferation of the gastric mucosa are the crucial events for malignancy. These results argue for a greater impact of host factors in carcinogenesis and call for the need of further studies with other *H. pylori* strains and in different hosts.

Giannakis et al. studied the possible differences in *H. pylori* strains during tumorigenic progression [10]. In a span of 4 years, of a group of chronic atrophic gastritis patients, a single individual progressed to GC and his colonizing *H. pylori* were recovered, characterized, and compared. It was determined that, by the time chronic atrophic gastritis developed, a single strain dominated the host's microbiota. Interestingly, marked differences were observed between the chronic atrophic gastritis-associated *H. pylori* isolate designated as Kx1 and the cancer-associated isolate Kx2. Interestingly, Kx1 was a significantly better colonizer, while Kx2 showed a greater capacity to invade cells and to adapt to the intracellular habitat. Moreover, Kx2 regulated the expression of metabolic pathways and tumor suppressor genes in a distinct manner from Kx1. These results suggest that the bacterial–host cell interaction is a dynamic coevolving relationship that provides an intracellular microhabitat to *H. pylori* but may also affect the risk of malignant

transformation. The authors speculate that during progression from chronic atrophic gastritis to cancer, such differences could be significant contributions to initiation and to progression and maintenance of tumorigenesis, respectively. They also claim that these observations may be in accordance with the cancer stem cell hypothesis. *H. pylori* intracellular invasion, although facultative, if occurring in long-living gastric stem cells, could contribute to gastric tumorigenesis due to the bacteria's capacity to adapt to intracellular residency but also to influence stem cell biology. This provocative theory constitutes a potential novel form of tumor initiation.

H. pylori-induced Changes in Epithelial Cell Signaling

Knowledge of the mechanisms that lead to *H. pylori*-induced gastric damage is crucial for both general understanding of the gastric carcinogenic process as well as for the development of prevention strategies and treatment. Several recent studies focused on the intracellular signaling pathways affected by bacterial–cell host interactions and explored host genetic (or epigenetic) changes occurring due to bacterial infection and chronic inflammation. Snider et al. demonstrated for the first time a CagA-independent stimulation of motility in *H. pylori*-infected AGS cells [11]. This effect was dependent on the bacteria type IV secretion system (TFSS) and was mediated by JNK activation occurring through β1-integrin and Src signaling. Importantly, a combination of CagA-dependent and CagA-independent signaling was required to stimulate cancer cell motility. Although both were necessary, neither was sufficient by itself to alter the phenotype. These data demonstrate that TFSS may play a more important role in host cell physiology than just the delivery of CagA from the bacterium into the host cell cytoplasm. Further studies are needed to assess the scope of CagA-independent signaling and to identify additional host cell players that participate in cell motility and contribute to GC progression.

Another cellular phenotype associated with carcinogenesis is altered apoptosis. Two publications studied differently the mechanisms of *H. pylori*-induced apoptosis. Minohara et al. evaluated the effect of bacterial properties, in particular the presence of the *cag* pathogenicity island (PAI), on apoptosis [12]. They showed that, although *cag*PAI-negative strains induced apoptosis, the expression of *cag*PAI promoted apoptosis more rapidly and increased DNA fragmentation in gastric epithelial cells. These results suggest that the genotype of infecting bacteria may impact the rate of apoptosis and therefore the carcinogenic process. On another work, it was demonstrated that the major *H. pylori*-induced apoptotic pathway in GC cells is the mitochondrial pathway and requires the activation of caspases-3 and −9 [13].

It has been described that *H. pylori* impairs the E-cadherin-β-catenin cell adhesion complex, leading to aberrant activation of β-catenin in an animal model, and thus is involved in precancerous intestinal metaplasia progression [14]. It was now demonstrated that deregulation of β-catenin signaling is dependent on the EPIYA-region of CagA (CagA-multimerization sequence) [15]. Although this region is variable among CagA of *H. pylori* strains isolated in Western and Eastern countries, activation of β-catenin was mediated by a 16-amino-acid sequence that is conserved among the two geographic subgroups. Finally, it was also shown that β-catenin deregulation did not depend on CagA tyrosine phosphorylation.

MMPs (matrix metalloproteinases), because they can digest the extracellular connective tissue, have the potential to disrupt gastric stroma and promote invasion [16]. Pillinger et al. reported MMP-1 secretion in *H. pylori*-stimulated gastric epithelial cells [17]. The observed stimulation involved both CagA-dependent and CagA-independent mechanisms and was mediated by ERK and inhibited by p38. Moreover, the pathways through which *cag*A+ and

*cag*A− strains activated ERKs were at least partially distinct. Finally, it was shown that CagA and in particular the EPIYA motif was required for optimal ERK activation and MMP-1 secretion.

Epithelial cell proliferation and reduced apoptosis are important factors in the transformation of epithelial cells to cancerous cells. The epidermal growth factor receptor (EGFR) is activated in response to epithelial injury and promotes epithelial repair processes, and EGFR transactivation has been linked to cell migration and inhibition of apoptosis [18]. It is also known that *H. pylori* induces EGFR activation [19]. Recently, it was shown that *EGFR* promoter activity increases due to *H. pylori* stimulation of the activator protein (AP)-1 transcription factor [20]. Exposure of gastric cells to *H. pylori* resulted in increased expression and phosphorylation of EGFR, ERK 1/2 phosphorylation, and increased levels of reactive oxygen species (ROS).

Initiating events that lead to GC are still not very well understood. A recent publication investigated some of its aspects comparing gastritis, metaplastic, and GC biopsies [21]. The authors observed a significant increase of STAT3 and ERK 1/2 signaling in *H. pylori*-dependent gastritis, an effect further enhanced by the presence of CagA. Coincidently, expression of STAT3-activator interleukin (IL)-6 was also augmented, indicating that it could promote STAT signaling. Activation of STAT3, but not of ERK 1/2, was achieved to a lesser extent by *H. pylori* CagA− strains, suggesting that STAT signaling may be driven by other bacterial factors. Such differential activation could, in part, explain the increased predisposition to GC associated with *H. pylori* CagA+ strains, particularly since increases in both STAT3 and ERK 1/2 activation could drive epithelial cell turnover. Finally, a strong up-regulation of another member of the IL-6 family, IL-11, was reported but only in adenocarcinomas. Unlike IL-6, this was not observed in gastritis, suggesting that it was not driven by *H. pylori* infection. Nonetheless, in vitro studies showed that IL-11 may be important later on GC development by maintaining mitogenic activity, as it also activated STAT3 signaling and increased proliferation of MKN28 cells.

Transformation of normal gastric mucosa to intestinal metaplasia is associated with loss of Sonic hedgehog (Shh) expression and aberrant expression of CDX2 (an intestine-specific transcription factor) [22]. It was now demonstrated that *H. pylori* eradication prior to development of incomplete intestinal metaplasia improved corpus gastritis, reversed the loss of Shh and reduced the expression of CDX2 [23]. Reversibility of *H. pylori*-correlated changes depended on the severity of such changes prior to eradication. Additionally, serum levels of pepsinogen I before *H. pylori* eradication were significantly lower in patients that eventually did not show improvement compared to patients that showed improvement, underlining that it may be a useful predictor of improvement as well as a marker for higher GC risk.

H. pylori-induced Epigenetic Changes in Epithelial Cells

Epigenetic changes, as genetic alterations, are involved in cancer development and progression. Promoter methylation is an event that can lead to gene silencing [24]. This mechanism is particularly relevant in cancer since it can interfere with the expression and activity of tumor suppressor genes. Accumulating evidence indicates that CpG island hypermethylation may be linked to *H. pylori* infection. This hypothesis was confirmed in a study by Kaise et al. [25]. The authors showed that rates of promoter methylation of *p16*, E-cadherin (*CDH-1*), and death-associated protein kinase (*DAPK*) were significantly higher in noncancerous gastric mucosa of GC patients than in controls, and this was independent of other parameters. Similar results were obtained in another study that analyzed *CDH1*, *p16*, *APC*, *MLH1*, and *COX2* promoters in non-neoplastic gastric mucosa of infected patients

[26]. Moreover, the authors demonstrated that *H. pylori* eradication significantly decreased *CDH1*, *p16*, and *APC* methylation and completely eliminated *COX2* methylation. In contrast, *MLH1* methylation did not change with treatment and seemed to occur later, simultaneously with intestinal metaplasia. Finally, it was shown that epigenetic changes were not uniformly distributed throughout the stomach and involved both normal and metaplastic epithelium.

In two publications, Chan et al. studied the contribution of IL-1 to promoter hypermethylation [27,28]. First they demonstrated that *IL-1* promoter polymorphisms were associated with increased methylation and *H. pylori* infection and described a synergistic effect between *IL-1* polymorphisms and presence of the bacteria to increase the frequency of methylated genes. Later on, they further tested these evidences in GC cell lines by treating cells with IL-1β and studying its effect on the E-cadherin promoter (used as a marker of methylation). Induced *CDH1* promoter methylation was observed in all cell lines upon treatment, a similar result to the one obtained with coculture with *H. pylori*. Moreover, this effect was reversed with IL-1ra treatment. The authors suggest that *H. pylori* infection and *IL-1* polymorphisms, via the increased production of IL-1β, predispose to gastric carcinogenesis by CpG island methylation of tumor suppressor genes.

Further knowledge on gene methylation and *H. pylori* infection was published this year. It was observed that *CDH1* methylation was greater in *H. pylori-positive* patients, and especially high in those with enlarged gastric body folds [29]. The mechanism of infection-induced methylation was also analyzed and the results obtained suggest that inflammatory cytokines and growth factors associated with *H. pylori* infection may induce *CDH1* methylation. Similar to descriptions from other groups, a significant decrease in methylation occurred after *H. pylori* treatment. Thus, the authors propose that *H. pylori*-infected individuals with enlarged folds are particularly good candidates for the prevention of GC through the use of antibiotics to eradicate the bacteria.

Overall, *H. pylori* infection seems to be a major environmental factor involved in CpG island hypermethylation. Significantly, *H. pylori* eradication could be used to reverse hypermethylation in noncancerous gastric mucosa and hypermethylation could constitute a predictive marker of GC risk. However, and despite of its obvious potential, no immunohistochemical correlation has yet been described between *H. pylori*-induced gene methylation and loss of protein expression, and so its true value may not be quite significant.

H. pylori and Host Immune Response

The functional interaction of *H. pylori* infection with the host immune response, especially T cells, in gastric carcinogenesis is not yet fully elucidated. Characterization of such interaction showed that *cag*A+ *H. pylori* infection was associated with Th1-mediated cellular immunity in earlier stages of GC, while Th2-mediated humoral immunity dominated the advanced stages [30]. Moreover, among immune responses associated with *cag*A+ *H. pylori* infection, local immunity was predominant over systemic immunity and polarization of Th cells mediated immune response associated with progression of gastric pathologies.

The possibility of occurrence of an altered immune response to *H. pylori* in GC patients compared to asymptomatic individuals was assessed by Lundin et al. [31]. When stimulated by *H. pylori*, T cells from both peripheral blood and gastric mucosa of GC patients produced high amounts of IL-10, while this production was low in *H. pylori*-infected asymptomatic individuals. Thus, it was proposed that increased production of suppressive cytokine IL-10 in *H. pylori*-infected GC patients may lead to a decreased cytotoxic antitumor T-cell response in the stomach, which may contribute to tumor progression. These are interesting

results, but the small number of cases enrolled in this study highlights the needed for further investigation.

Gastric Mucosa-associated Lymphoid Tissue Lymphoma

H. pylori infection plays a decisive role in the development and progression of gastric mucosa associated lymphoid tissue (MALT) lymphoma. Eradication of *H. pylori* is the first choice in treating localized stage I gastric MALT lymphoma with *H. pylori* infection [32,33]. A recent Japanese study showed the outcomes of 74 patients with the localized gastric MALT lymphoma after eradication therapy alone, where 56 patients showed complete remission at the time point of 12 months posteradication [34]. In addition, none of these patients, who did not undergo any second-line therapy, exhibited disease progression at their median follow-up period of 46 months [34]. In a large European international series study, 94% of patients (102 of 108) with stage I gastric MALT lymphoma had a favorable disease course based on a follow up of a median of 42.2 months; 35 (32%) went into complete remission, whereas in 67 patients (62%) the minimal histologic residuals remained stable [35]. These reports have confirmed that *H. pylori* eradication provides a favorable long-term prognosis, with a genuine chance of a cure or at least of long lasting complete remission in the majority of patients. Thus, a watch- and-wait strategy with regular endoscopies and biopsies is a justified approach for treating patients with localized gastric MALT lymphoma, even those with minimal residual disease. Nevertheless, careful monitoring for the development of GC is recommended during the follow up, given recent case reports on metachronous stomach cancers after eradication therapy for gastric MALT lymphoma [36,37].

In cases where the *H. pylori* status is negative, single-agent or multiagent chemotherapy or radiotherapy is currently applied with good results if the lymphoma does not reveal regression after eradication therapies, or if the lymphoma is in stage II [32,33]. Additionally, surgical resection is a highly curative treatment for gastric MALT lymphoma when coupled with a long-term follow-up period of 11.5 years [38].

MALT lymphoma is genetically characterized by four chromosome translocations. The t(11;18)(q21;q21) generates a functional API2 (apoptosis inhibitor 2)-MALT1 fusion transcript, whereas the other three translocations involve translocation to immunoglobulin gene (IGH) loci and consequently increased expression of BCL10 (1p22), MALT1 (18q21), and forkhead box protein P1 gene (FOXP1) (3p14). Nakamura et al. recently reported that t(11;18)/API2-MALT1 was frequent (n = 87, 21%) in Japanese MALT patients [39], which was compatible with previous large cohort of unselected cases in Western countries (15–24%), and thus the frequency of 20% likely represents its true incidence in gastric MALT lymphoma. The clinical impact of t(11;18)/API2-MALT1 has been well characterized: gastric MALT lymphomas with this translocation are usually unresponsive to *H. pylori* eradication, often present with advanced disease, and rarely undergo high-grade transformation, which have been further confirmed in studies during the last year [32,39–43]. In a recent Japanese study, 86 cases of gastric MALT lymphoma treated with *H. pylori* eradication therapy were classified into three groups based on responsiveness to therapy and API2-MALT1 status; responders without API2-MALT1 (n = 56, 65%), nonresponders without API2-MALT1 (n = 16, 19%), and nonresponders with API2-MALT1 (n = 14, 16%) [42]. All but one of the responders attained complete remission with the eradication therapy. For the nonresponders, second-line treatments exclusively resulted in complete remission, and even when second-line therapy was declined, the disease did not progress in most non-responders. Notably, in one nonresponder with API2-MALT1 without any second-line treatment, lymphoma metastasized to the lung [42], suggesting that careful monitoring for disease progression is necessary for gastric MALT lymphoma carrying API2-MALT1.

Array-based comparative genomic hybridization demonstrated no significant genetic alterations in the t(11;18)(q21;q21)-positive MALT lymphoma [41], thus it is considered to have a good prognosis. In contrast, gastric t(11;18)(q21;q21)-negative MALT lymphoma with *H. pylori* independency exhibits numerous genomic abnormalities similar to those seen in diffuse large B-cell lymphoma, which is characterized by a clinically advanced form of the disease and shows an increase in the large cell component [32,41,42]. Overall, these data suggest that acquired genomic imbalances may play a more important role in the *H. pylori*-independent than the *H. pylori*-dependent stage of gastric MALT lymphoma [41].

In contrast to t(11;18)(q21;q21), there is little information on the clinical features of gastric MALT lymphomas bearing other translocations or numerical changes. During the last year, these genetic abnormalities were also intensively studied [39,44,45]. In the Japanese population, IGH-involved translocations (FOXP1, BCL2, or an unknown partner) were rare, with 6% of gastric MALT lymphoma [39] and cases with t(14;18)/IGH-BCL2 responding well to *H. pylori* eradication therapy [44]. Polysomy of *MALT1* was detected in 14–25% of the gastric MALT lymphoma cases [39,45] and the presence of extra copies of *MALT1* was significantly associated with progression or relapse of the disease, and was an independent adverse prognostic factor for event-free survival [39]. Further longer follow-up studies with a large number of cases are necessary to clarify whether this numerical change has a major influence on the prognosis of gastric MALT lymphoma.

Besides the cytogenetic analyses, there were several intriguing immunohistochemical studies. Toll-like receptor (CTLR) 4 expression was exclusively localized on the surface of MALT lymphoma cells but not other lymphomas (chronic lymphocytic leukemia and mantle cell lymphoma) infiltrating the stomach [46], suggesting a pathogenic role and a therapeutic target of the receptor for gastric MALT lymphoma. Low-grade MALT lymphoma with superficial gastric involvement showing the BCL2-positive and p53-negative immunophenotype disappears after successful treatment of *H. pylori* infection [47].

As for the relationship between MALT lymphoma and *H. pylori* factors, the presence of anti-CagA IgG antibodies were confirmed to be a risk factor for lymphoid follicle development in patients with gastritis [48]. Immunohisto-chemistry analyses showed that cytoplasmic heat shock protein B (HspB) immunostaining was observed in groups of neoplastic cells of MALT lymphoma [49]. HspB is one of the dominant proteins of *H. pylori* and is being considered as a potential candidate for subunit vaccines, and the data suggest a possible involvement of HspB in the pathogenesis of *H. pylori*-related development of MALT lymphoma.

Finally, *Helicobacter heilmannii* is known to induce gastric low-grade MALT lymphoma in mice model. In addition, epidemiologic studies have also revealed that there is a high rate of MALT lymphoma in patients with *H. heilmannii* infection compared with the rate in those infected with *H. pylori*. During the last year, its pathophysiologic mechanism was better clarified using a *H. heilmannii* infected C57BL/6 mice model [50]. Enhanced immuno-reactivity of vascular endothelial growth factor (VEGF)-A and -C was observed in areas encircled by increased parietal cell apoptosis, indicating that it has pathophysiologic relevance in both angiogenesis and apoptosis of MALT lymphoma formation. The clinical studies for investigating the roles of *H. heilmannii*, especially in *H. pylori*-independent cases, will provide further information of the mechanisms of gastric MALT lymphoma.

Gastric MALT lymphoma is still an interesting human and experimental model that will continue to give us important insights into lymphomagenesis.

Animal Model of CagA-induced Malignancy

H. pylori strains possessing the *cag*A gene are associated with an increased risk of gastric malignancy compared with *cag*A-negative strains. *cag*A has been reported to interact with various target molecules in the host cells; the best studied is the cytoplasmic Src homology 2 domain of Src homology 2 phosphatase (SHP-2). In vitro studies have shown that phosphorylated CagA-induced signaling via SHP-2 is a crucial step in *H. pylori*-induced carcinogenesis. Ohnishi et al. reported the first proof that CagA protein alone is capable of inducing tumors in vivo using a CagA transgenic mice model [51]. They constructed mice that expressed either wild-type CagA protein or phosphorylation-resistant CagA protein, either predominantly in the stomach or throughout the body. Approximately 10% of the wild-type CagA transgenic mice showed gastric hyperplastic polyps and about 1% of them developed gastric or small intestinal cancers after 72 weeks of observation irrespective of whether the CagA was targeted specifically to the stomach or throughout the body. Systemic expression of wild-type CagA further induced leukemias in a small number of mice. Importantly, such pathologic abnormalities were not observed in transgenic mice expressing phosphorylation-resistant CagA, confirming the importance of phosphorylated CagA-induced signaling in *H. pylori*-induced carcinogenesis. This elegant study showed evidence that indicated the role of CagA as a bacterium-derived oncoprotein; however, it was noted that only a small minority of the transgenetic mice developed tumors, indicating that CagA itself is only weak an oncoprotein and that additional factors likely contribute to carcinogenesis in *H. pylori* infections.

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