REVIEW

# *Helicobacter pylori* infection and gastric carcinoma

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## Abstract

*Helicobacter pylori* infection is considered to be the main cause of gastric cancer and the most frequent infection-induced cancer. *H. pylori* is a heterogeneous species which can harbour pathogenic factors such as a cytotoxin, a pathogenicity island (*cag*) encoding a type 4 secretion system, and the first bacterial oncoprotein, CagA. This oncoprotein appears to be involved in the carcinogenic process in addition to the inflammation generated. This process may concern either local progenitors *via* an epithelial–mesenchymal transition, or recruited bone marrow–derived mesenchymal cells. There are also environmental factors such as iron deficiency or high-salt diets which interact with the bacterial factors to increase the risk of gastric cancer as well as genetic polymorphism of certain cytokines, e.g. IL-Iβ. Recent data suggest that a break in coevolution of a particular phylogeographic lineage of *H. pylori* and its usual host may also be a risk factor. Studies are currently being performed to assess the feasibility of organized *H. pylori* eradication programmes to prevent gastric cancer.


**Keywords:** CagA oncoprotein, cancer stem cells, carcinogenesis, epithelial–mesenchymal transition, prevention, VacA cytotoxin
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## Introduction

Among the cases of cancer which could be attributed to infection, in the world in 2008, i.e. 16.1% of new cases (about 2 million out of 12.7 million) according to the International Agency for Research on Cancer, *Helicobacter pylori* infection was the leading cause and represented 5.5% of the total number of cases, while the other main agents were viruses [1].

*H. pylori* was discovered relatively recently, in 1982, and has proved to be the cause of gastric and duodenal ulcers [2]. For this discovery, Warren and Marshall were awarded the Nobel Prize for medicine in 2005.

However, *H. pylori* infection is also considered to be the main risk factor of gastric cancer development, namely gastric carcinoma and gastric mucosa-associated lymphoid tissue lymphoma [3] (Fig. 1). Here we will only consider the former, while the latter will be discussed in another article. We will review the characteristics of *H. pylori* and the consequences of the infection, the risk factors other than *H. pylori* for cancer development and the current status of prevention of gastric cancer.

## *Helicobacter pylori*

*H. pylori* is an epsilonproteobacterium and a member of the *Helicobacteraceae* family, separated from *Campylobacteraceae* in 1989 [4]. Helicobacters are classified into two types according to their customary niche: gastric and enteric. The *Helicobacter* species adapted to humans is *H. pylori* and is a gastric *Helicobacter*. Others originating from pets or food animals can rarely be found in humans as zoonotic bacteria.

*H. pylori* is a very heterogeneous species but characteristics important for colonization and pathogenesis are found in most of the strains. *H. pylori* harbours various adhesins, the most important being BabA and SabA, which allow it to colonize the epithelial layer, mainly in the antrum. Indeed, despite living in the stomach, *H. pylori* is relatively acid sensitive. It can grow at pH 5 but does not grow and only survives at pH 4. A key factor is its production of urease, which is quantitatively important



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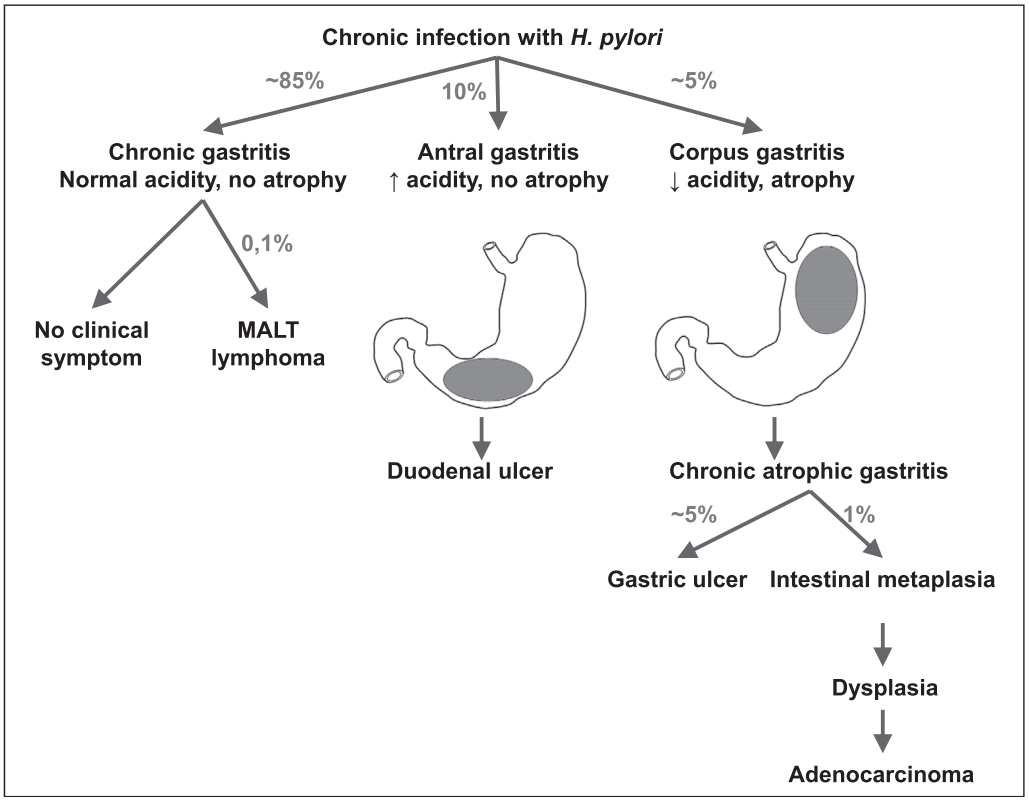


**FIG. 1.** Various consequences of *Helicobacter pylori* infection.

and allows it to buffer its microenvironment by breaking down the low amount of urea present in the gastric mucosa, producing ammonia [5].

Once colonization is established, the infection can last for decades and can even be lifelong. The basic lesion observed in all cases is gastritis, i.e. an infiltrate of lymphocytes and polymorphs in the gastric mucosa. In some individuals, and especially youngsters, it may even take on the aspect of lymphoid follicles, normally absent in the stomach [6].

Among the pathogenic factors is a cytotoxin named VacA. There is a polymorphism of the *vac*A gene, and according to the allele present, varying amounts of the toxin can be produced influencing the outcome [7]. A pathogenicity island is also present in about half of the strains. Among the 30 genes present in the island, some code for a type 4 secretion system (T4SS), which is similar to a syringe allowing the introduction of bacterial molecules in the epithelial cell. The most important of these compounds is CagA, considered to be the first bacterial oncoprotein. Once in the epithelial cell, CagA is phosphorylated by cellular kinases and then interacts with a number of signalling pathways [8]. CagA is also heterogeneous with regard to the number and type (A, B, C, D) of phosphorylation motifs present. Types C and D have been associated with the highest risk of cancer [9].

Other *H. pylori* molecules can also be introduced into the epithelial cells, such as muramyl dipeptide (MDP), which is part of the peptidoglycan cell wall of the bacterium. MDP is detected by the intracellular NOD receptors which activate the NF-κB pathway with production of IL-8 and attraction of inflammatory cells [10].

## Oncogenic consequences of *H. pylori* infection

Gastric adenocarcinoma is a heterogeneous cancer. First, it is necessary to distinguish the tumours arising from the gastric proximal stomach (cardia), as most of them are not linked to *H. pylori* infection from those found in the distal part of the stomach. Among tumours from the distal stomach, on the basis of histology, it is usual to differentiate two types of cancer lesions: the intestinal type and the diffuse type according to the Lauren classification [11].

Intestinal type cancer is the most frequent. It corresponds to a slow evolution of the gastric mucosa which becomes atrophic; then intestinal metaplasia appears, followed by dysplasia and ultimately *in situ* gastric carcinoma and metastatic carcinoma (Fig. 2). This is the so-called Correa cascade, which was described before *H. pylori* was discovered and appears late in life [12].

 

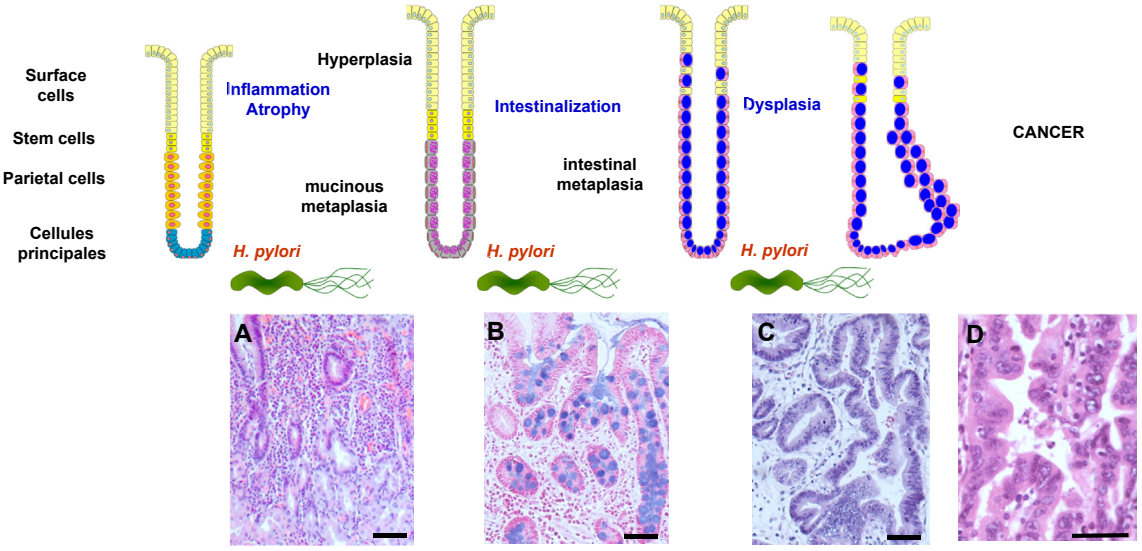


**FIG. 2.** Cascade of histologic changes induced by *Helicobacter pylori* at level of gastric mucosa.

The other histologic type of gastric carcinoma is the diffuse type, which does not show these different steps and usually occurs early in life. Furthermore, mutations in the E-cadherin gene (*cdh*1) are found in about 30% of the cases. The expression of this molecule is then inhibited at the adherent cellular junctions, leading to invasive tumours.

Besides this histologic classification, a molecular classification has recently been proposed. It can identify gastric carcinoma due to the Epstein-Barr virus, which represents about 10% of the cases. The others are microsatellite unstable tumours showing elevated mutation rates including mutations in targetable oncogenic signalling proteins, genomic stable tumours and tumours with chromosomal instability [13].

The role of *H. pylori* infection in the carcinogenic process was first considered to be indirect *via* the long-term inflammation that is induced. The Th1 type immune response leads to apoptosis of the gastric epithelial cells [14] and to a cell proliferation to compensate for the cell loss [15]. The important production of oxygen free radical species leads to errors during mitosis and an accumulation of mutations [16]. This process may be reinforced by the fact that *H. pylori* impairs DNA mismatch repair in gastric epithelial cells [17].

More recent data have shown that *H. pylori* may also have a direct carcinogenic effect *via* the CagA protein. CagA interacts with proteins of the tight junctions: ZO-1, JAM and adherent junctions, with E-cadherin leading to a destabilization of these junctions and activation of β-catenin [18]. This effect would be due to the interaction of CagA with the PAR1 kinase (partitioning-defective 1 microtube affinity-regulating kinase MARK) involved in the cytoskeleton structure and cell polarity [19].

Other effects of CagA appear after its phosphorylation. It then interacts with the SHP2 phosphatase which inhibits Src dephosphorylation, FAK which regulates the focal adherence plaques to the cellular matrix, and also c-Met, MAPK and other actin cytoskeleton regulators [8]. The outcome is the change in phenotype of the epithelial cell, described as the "hummingbird" phenotype (Fig. 3). We showed that this change indeed corresponds to an epithelial–mesenchymal transition, with an increase in mesenchymal cell markers and a moderate decrease in epithelial cell markers. Furthermore, these cells express a high level of CD44, which is a marker of gastric cancer stem cells (CSC) and which present CSC-like properties [20,21].

In the Mongolian gerbil model it was possible to induce the cascade of events leading to gastric carcinoma by infecting the animals with a CagA-positive strain, while these modifications were not observed with the CagA isogenic mutant, confirming the oncogenic role of CagA [22].

The final proof of the oncogenic potential of CagA came from the design of a transgenic mouse model expressing CagA either ubiquitously or in gastric tissue only. It was shown that without *H. pylori* infection, these mice could develop hyperplastic polyps but also gastric adenocarcinoma in a limited number [23].

## Origin of cancer stem cells in gastric cancer

As mentioned before, *H. pylori* is able to induce an epithelial–mesenchymal transition which generates cells with CSC properties, and therefore the cancer may rise from local

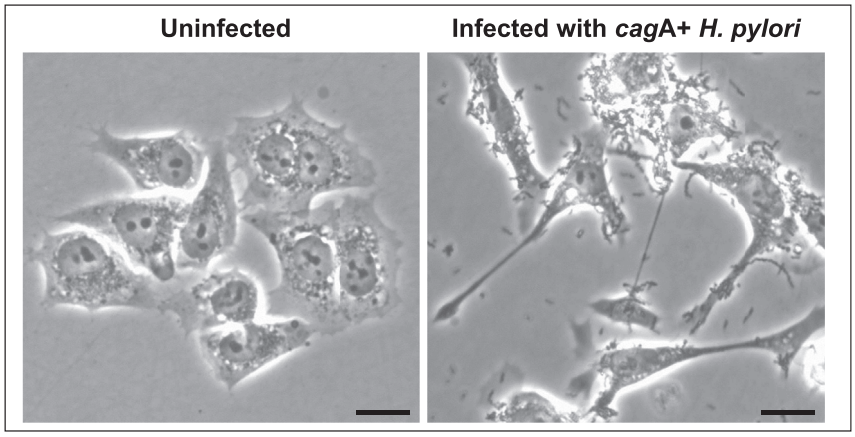


**FIG. 3.** Appearance of cells infected with CagA-positive *Helicobacter pylori* ("hummingbird" phenotype) and controls.

progenitor cells. However, interestingly, data indicate that it may also develop from bone marrow–derived cells (BMDC), which are mesenchymal stem cells. After the work of Houghton *et al.* [24] using *Helicobacter felis* in an experimental mouse model, our group studied this phenomenon in depth. The concept is that because of *H. pylori* infection and intense inflammation in the gastric glands, the local regeneration process is insufficient. As a result of chemotactism, there is a recruitment of circulating mesenchymal type BMDC to home within the gastric mucosa and contribute to tissue regeneration, but because the infectious process is still present, these cells cannot correctly differentiate to repair the gastric mucosa and may become CSC. We used a mouse model of C57Bl/6 mice irradiated and transplanted with bone marrow from transgenic mice expressing green fluorescent protein. The chimeras were infected with different strains of *H. pylori* and *H. felis* as a control and humanely killed after 15, 35, 55 and 75 weeks to look at gastric pathology and BMDC recruitment. We could observe the usual gastric lesions of mice beginning with hyperplasia, atrophy, mucinous and pseudointestinal metaplasia evolving in aged animals towards dysplasia and gastric intraepithelial neoplasia (Fig. 4). In addition, after a year of infection, we showed that about a quarter of the gastric intraepithelial neoplasia lesions were green fluorescent protein positive, indicating that mesenchymal BMDC had indeed colonized the glands and were at the origin of the lesions [25].

Further *in vitro* studies using cell culture models showed that the capacity to recruit mesenchymal stem cells was indeed variable among *H. pylori* strains and not linked to CagA. The mechanism involved was the production of chemokines including TNF-α [26]. It was also possible to show that recruited cells fused with gastric epithelial cells [27].

## Risk factors other than *H. pylori* infection involved in gastric cancer

It is now accepted that the characteristics of the infectious strain are the most important factors in the development of gastric carcinoma, but, like in other infections, there are environmental factors and genetic factors which predispose to or protect from this outcome and which furthermore interact with some pathogenic factors of the strain, e.g. CagA.

### Genetic factors

Rare cases of hereditary gastric carcinoma exist such as the Lynch syndrome, the hereditary nonpolyposis colorectal cancer

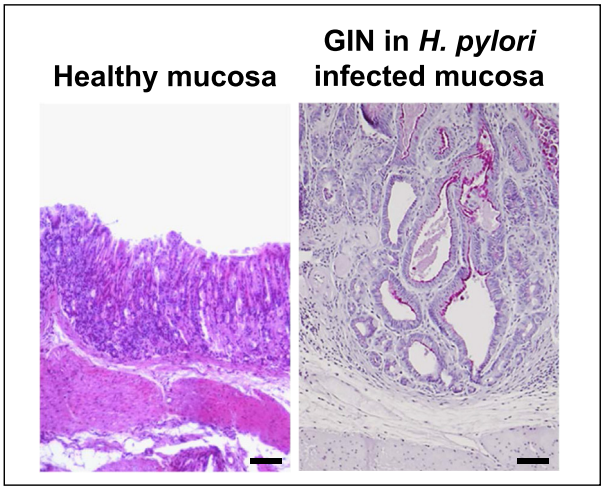


**FIG. 4.** Gastric intraepithelial neoplasia induced by *Helicobacter pylori* infection in mouse model.

 

syndrome, or the familial adenomatous polyposis. It may be that a *H. pylori* infection speeds up the carcinogenetic process.

Recently, following the high-throughput sequencing of the human genome, it was possible to identify single nucleotide polymorphisms which are associated with a higher risk of developing gastric adenocarcinoma. The main one concerns IL-1β and its receptor [28]. Others include TNF-α, IL-8, IL-10 and COX2.

### Environmental factors

Environmental factors are mostly related to diet. A diet including antioxidants, e.g. ascorbic acid and polyphenols, is protective against gastric carcinoma [29]. On the other hand, meat consumption, which induces nitrosamines, is a risk factor [30], as is use of tobacco products, as for many other cancers.

High dietary salt intake is a risk factor for gastric cancer and may have a synergistic effect. There was a higher CagA transcription *in vivo* in the animals submitted to this diet [31]. Iron deficiency also increases cancer risk by increasing the virulence phenotype of CagA-positive *H. pylori,* as shown by Noto *et al.* [32].

### Ancestral origin of the *H. pylori* strains

An interesting concept has been proposed to explain the evolution of *H. pylori* infection towards gastric cancer in some individuals.

*H. pylori* strains can be categorized in 11 groups according to their phylogeographic origin determined by multilocus sequence typing, which allows a migration trace of modern humans beginning when they went out of Africa 60 000 years ago [33,34].

When human ancestry was compared to *H. pylori* ancestry in Colombia where different populations are living, it was shown that the interaction between African *H. pylori* ancestry and populations of African ancestry was relatively benign, while it was deleterious in populations with substantial Amerindian ancestry [35].

This rupture in coevolution shapes the risk of cancer development. This nice result remains to be confirmed in other types of populations.

## Prevention of gastric cancer

There are now many data indicating the role of *H. pylori* infection in the development of most gastric cancer cases. Accordingly, it has raised the possibility of prevention of this disease by eradicating *H. pylori.* Nevertheless, such an approach needs to be studied carefully in order to appreciate the benefits and the risks [36].

We do not have many studies showing the benefit of eradication on gastric carcinoma occurrence. Wong *et al.* [37] in China showed that gastric carcinoma could be prevented if no premalignant lesions were present at the time of eradication, while there was a limited efficacy in the case of premalignant lesions. Given that *H. pylori* infections last for decades, it is understandable that in the case of premalignant lesions, mutations may have occurred and so it is not possible to return to a zero risk.

To obtain suitable data concerning the impact of *H. pylori* eradication with gastric carcinoma development as the outcome, a large trial is currently taking place in China: the Linqu County trial. A population of 184 000 subjects aged 25 to 54 years were tested for *H. pylori* by urea breath test, and 57.6% were positive. They were randomized by village in two groups of about 45 000 receiving either a quadruple therapy or bismuth and omeprazole as a comparator. The eradication rate was 73% in the former and 15% in the latter. These subjects will now be followed for 7 years [38,39].

Other eradication trials in the general population have been carried out, e.g. on Matsu Island, Taiwan (5000 inhabitants), but with no control group. Using a historical comparison, the researchers were able to show a 25% decrease in gastric cancer and a 67% decrease in peptic ulcer disease after 5 years [40].

There are, however, some risks induced by an organized screening and treatment of *H. pylori* infection, the main one being the risk linked to antibiotic treatment. A relationship between antibiotic consumption and bacterial resistance has been shown at the population level. Concerning *H. pylori,* a study carried out in Europe highlighted an association between the consumption of long-acting macrolides and *H. pylori* resistance to clarithromycin, as well as between fluoroquinolone consumption and *H. pylori* resistance to levofloxacin in the community [41]. At the individual level, it is also clear that in the case of failure of a clarithromycin-based regimen, approximately 60–70% of the strain test resistant to clarithromycin [42]. It is also important to consider resistance induced in other bacteria, especially in the faecal flora. Very few studies have been carried out, but they show a dramatic increase in resistance of a number of bacteria, and resilience may not always occur after stopping the treatment [43–45]. In addition to the selection of resistant bacteria, antibiotics can modify the gut microbiota, with possible harmful consequences.

Other possible negative effects of *H. pylori* eradication have been pointed out. The first concerns autoimmune diseases and is based on a mouse model [46]; however, it appears that this would be relevant if an eradication attempt is carried out very early in life [46], which is not the case. The second concerns gastroesophageal reflux; however, while a negative association was found between the prevalence of *H. pylori* and the severity

of gastroesophageal reflux, there is much controversy regarding a possible causal relationship [47].

## Conclusion

There are numerous arguments showing that *H. pylori* infection can cause gastric cancer. This is the main cancer induced by infection worldwide. A bacterial oncoprotein, CagA, has been identified. Prevention of gastric cancer is now possible, but it is important to weigh the benefits and risks of this approach.

## Transparency declaration

All authors report no conflicts of interest relevant to this article.

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