

REVIEW

Unraveling CagA's potential: A promising frontier for gastric cancer

Asma Boudouaia-Ouali^{1,*}, Majda Dali-Sahi^{1,2}

¹Department of Biology, University of Tlemcen, Tlemcen 13000, Algeria

²Analytical Chemistry and Electrochemistry Laboratory, University of Tlemcen, Tlemcen 13000, Algeria

ABSTRACT

In the relentless pursuit of conquering gastric cancer, researchers have uncovered a promising avenue: CagA, the captivating oncoprotein encoded by *Helicobacter pylori* (*H. pylori*). This review delves deep into the multifaceted role of CagA in the pathogenesis of gastric cancer, shedding light on its remarkable ability to manipulate signaling pathways and trigger the epithelial-mesenchymal transition (EMT) phenomenon, which underlies cancer invasion. By unraveling the mysteries of *H. pylori*'s influence on signaling pathways within gastric cells, this review presents cutting-edge insights into the pathogenesis of this devastating malignancy. Moreover, it unveils the fascinating relationship between *H. pylori* infection and extra-gastric diseases, offering a panoramic view of the broader impact of this microbe on human health. Beyond the realm of basic science, this exploration of *H. pylori*'s dark secrets holds the promise of revolutionizing the realm of gastric cancer prevention, diagnosis, and treatment. With CagA as the spotlight, the stage is set for a transformative journey towards conquering this devastating malignancy and embracing a brighter future in cancer therapeutics.

Key words: *Helicobacter pylori*, gastric cancer, oncoprotein CagA, epithelial-mesenchymal transition, vacuolating cytotoxin

INTRODUCTION

Gastric cancer, the fifth most common type of cancer, took the third place as the main reason for cancer-related deaths in 2018, accounting for more than 800,000 fatalities globally. Although developed nations are the ones where gastric cancer is diagnosed the most frequently, there are large geographic variations in the prevalence of gastric cancer. Eight times more cases of gastric cancer occur in East Asian nations than in North American ones, including Japan, China, and Korea. More than half of all cases of gastric cancer occur in these nations.^[1] The incidence of gastric cancer is 2.2 times higher in men than in women,^[2] making it a cancer that disproportionately affects men.


With nearly 4.4 billion infected individuals, or more than half of the global population, *Helicobacter pylori* (*H. pylori*).

infection is regarded as the most prevalent chronic bacterial infection in the world.^[3,4] More than half of the world's population has *H. pylori* colonized stomachs, and 20% of those people develop gastric ulcers even though most people show no symptoms. *H. pylori* is the first and only bacterial agent for which a connection with a cancerous pathology has been recognized since *H. pylori* was classified as a type I carcinogen by the International Agency for Research on Cancer.^[5] More seriously, the bacterium can cause adenocarcinomas or MALT-type lymphomas (mucosa-associated lymphoid tissue) in 3% of patients.

With four to unipolar flagella located in its distal part, this spiral-shaped Gram-negative bacterium is highly motile. The bacterium is between 0.5 and 1.0 μ m wide and 2.5 to 4.0 μ m long. It is a micro-aerophilic bacterium that two Australian researchers, Barry Marshall and J.

*Corresponding Author:

Asma Boudouaia-Ouali, Department of Biology, University of Tlemcen, Tlemcen 13000, Algeria. E-mail: soma_1308@yahoo.fr. <https://orcid.org/0000-0002-5059-0964>
Received: 5 August 2023; Revised: 12 August 2023; Accepted: 12 August 2023; Published: 21 September 2023
<https://doi.org/10.54844/gfm.2023.435>

 This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License, which allows others to copy and redistribute the material in any medium or format non-commercially, as long as the author is credited and the new creations are licensed under the identical term.

Robin Warren, isolated from human stomach extracts in 1984. They identified it as the primary culprit behind the huge percentage of gastric ulcers and gastroduodenal affections. Their discovery earned them the 2005 Nobel Prize in Physiology or Medicine.

Helicobacter comes in many different varieties, and *H. pylori* is thought to be the type that only affects humans. As a result, it is believed that *H. pylori* only lives in the human stomach. Therefore, it spreads from person to person and mainly affects young children.^[6] The estimated seroprevalence in kids rises with age, from 20% in kids under 6 to 37% in kids between 6 and 11 to 44% in kids between 12 and 18 years old.^[7] *H. pylori* reduces gastric acidity through its urease activity. It is capable of nesting on epithelial cells thanks to its flagella and attachment proteins like adhesins.

Because it resists peristaltic emptying of the stomach contents, it stays in the gastric mucosa.^[8] However, the ammonia that the bacteria produce damages the epithelial cells, leading to acute and then chronic gastritis (a condition in which the stomach lining is inflamed), which can result in burning feelings. It is important to keep in mind that the development of gastric oncogenesis requires the presence of *H. pylori*, but that it is not a requirement. Gastric adenocarcinoma develops slowly over many years. During organogenesis, tissue development, remodelling, and wound healing, the epithelial-mesenchymal transition (EMT) is seen physiologically; however, any deregulation could lead to carcinogenesis.^[9]

Cells lose their epithelial characteristics and acquire mesenchymal properties during the dynamic and reversible epithelial-mesenchymal transition. Complex architectural and functional changes are necessary for process. EMT may not always be a full cell lineage change because the change from one phenotype to another encompasses a wide range of intracellular and intercellular changes that are infrequently observed in their entirety. EMT-affected cells develop new plastic abilities, such as motility and an apolar fibroblastoid phenotype resembling mesenchymal cells; they also become resistant to apoptosis. They exhibit distinctive features such as the absence of epithelial elements like cell-cell junctions, organization of the actin cytoskeleton, polarized distribution of intracellular organelles, expression of mesenchymal markers, and production of invasive factors. A change in the cell phenotype enhances the migratory abilities, invasiveness, and apoptosis resistance of the cells. Additionally, *H. pylori* has been shown by Bessede to cause changes in gastric epithelial cells that resemble EMT and cancer stem cells.^[10] Therefore, in order to create fresh approaches to combat gastric cancer, it is crucial to comprehend the molecular mechanisms of *H. pylori*-induced EMT.

Additionally, EMT is linked to the induction of cancer stem cell traits that result in chemoresistance and tumour dormancy. By infiltrating macrophages, neutrophils, regulatory T cells, and natural killer cells, *H. pylori* infection significantly alters the gastric microenvironment and causes several inflammatory responses.^[11]

EMT in gastric cells is facilitated by inflammatory mediators released by gastric and infiltrating cells, including cytokines, chemokines, and metalloproteinases.^[12] Hydrochloric acid (HCl) in the stomach promotes further digestion to assist in removing nutrients farther into the gastrointestinal tract. Hyperchlorhydria causes antral gastritis to form more frequently, duodenal ulcer risk to rise, and cancer risk to decrease or disappear. Contrarily, a low acid secretion encourages the growth of pangastritis and raises the danger of gastric cancer. The most active urease known to date is the *H. pylori* urease. Its capacity to produce ammonia is much greater than that needed to keep the bacterium's cytoplasmic pH at or near 7.

Due to the urease activity of *H. pylori*, gastric acid is neutralized. It can nest on epithelial cells because of flagella and proteins that fix other proteins, such as adhesins. Because it resists peristaltic emptying of the stomach contents, it stays in the gastric mucosa.⁸ However, the ammonia that the bacteria produce damages the epithelial cells, leading to acute and then chronic gastritis (inflammation of the stomach lining), which may cause burning feelings. The likelihood of contracting *H. pylori* varies with age, ethnicity, and living circumstances,^[13–15] and most cases happen in children.^[16]

What about inherited gastric cancer, though? The discovery of germline mutations in the CDH1/E-cadherin gene in three families in 1998, mutations that were later described in families of various ethnic backgrounds,^[17] marked the first molecular manifestation of the idea of hereditary gastric cancer.

In subjects with (69%) compared to subjects without (44%) a family history of gastric cancer, *H. pylori* prevalence is higher, according to serology estimates.^[18] Based on host gene polymorphism, the E.M. El-Omar team examined gastric cancer risk. Gastric atrophy and hypochlorhydria were more prevalent (27%) in first-degree relatives of gastric cancer patients than in controls (3%), despite a similar incidence of *H. pylori* infection (63% *vs.* 64%) in three different populations: English, Americans, and Poles.^[19]

It follows that the role of *H. pylori* may also be augmented by a genetic propensity for cancer. E.M. El-Omar *et al.*^[20,21] first hypothesized and subsequent research has confirmed an association between polymorphism of the interleukin-1 (IL-1) gene and the

risk of gastric atrophy and cancer. One of the bacteria with the highest genetic polymorphism is the *H. pylori* species. The efforts of Dr Venter's team^[22] made it possible to sequence the genome of *H. pylori*, one of the first bacteria. Small size (about 1.6 Mb. 1.7×10^6 bp, 1590 coding sequences).^[23]

The *H. pylori* genome analysis reveals significant differences between strains of *H. pylori* as well as other Gram-negative bacteria. These differences are a result of *H. pylori*'s long isolation from other bacterial species^[24] and adaptation to the stomach. Geographical regions have a different impact on the genomic sequence of various strains of *H. pylori*.^[21,25] The genes necessary for the synthesis of urease, the cytotoxin VacA, the antigen CagA, and flagellins are found in the essential chromosomal regions. Only about 50% of the strains isolated in Europe or the USA have the CagA pathogenicity island and toxigenic alleles of the VacA gene, in contrast to the majority of the strains isolated in East Asia.

The pathogenicity island of *H. pylori* is a collection of 32 genes whose expression mitigates the harmful effects of allelic variability and serves as a gauge of the bacterium's virulence. Among the most investigated genes in the bacterial genome are the VacA, pathogenicity island, and CagA (cytotoxin-associated gene A) genes.

In conjunction with host factors affecting the inflammatory response, such as pro-inflammatory cytokines, *H. pylori* secretes the oncoprotein CagA, allowing the lesion to develop.^[26] Type IV "TFSS" secretion systems are used by *H. pylori* strains that are CagA-positive to begin delivering oncoproteins into the cytoplasm of host cells. This secretion system functions as a true syringe, allowing the bacterial CagA protein and peptidoglycan to enter the target gastric epithelial cell.^[27,28] The interaction of CagL, a component protein of SST4, with integrin 5B 1 is crucial for the translocation of CagA,^[29] which initiates the formation of a conduit connecting the bacterium and the host cytoplasm to deliver CagA.

A double membrane spanning channel, an outer pilus, and a cytoplasmic/intracellular complex are the three interconnected subparts of the device, which is made up of twelve proteins (VirB 1-11 and VirD4). The only T4SS protein substrate known to date that is translocated into host cells during infection is the CagA cytotoxin.^[30-32] Up to 20 host-binding partners for CagA have been discovered, and host cell kinases are located very close to CagA phosphorylation sites.^[33] Studies have shown that translocated CagA can activate complex host signaling pathways by using both dependent and independent mechanisms of CagA EPIYA phosphorylation.^[34] When CagA is introduced into gastric epithelial cells, it attaches to the inner leaflet of

the plasma membrane using one of two different mechanisms, depending on the cell's polarity state.

It is crucial for CagA to interact with the membrane in polarized epithelial cells because this region of CagA contains several basic amino acids, including those that make up the PS-binding K-Xn-RXR motif.^[34] However, in unpolarized cells, the C-terminal portion of CagA is primarily in charge of membrane binding.^[35]

Only patients with *H. pylori* infection (2.9% *vs.* 0%) developed CG during an endoscopic follow-up of 1,526 patients over an 8-year period in 2001, according to Uemura *et al.*^[36] Except for those who had a duodenal ulcer, patients who tested positive for *H. pylori* had a higher risk of developing cancer. This study provides concrete evidence of the actual risk of "positive *H. pylori*" gastritis for the first time. Another Japanese study demonstrates the significant increase in the risk of cancer recurrence in patients who received conservative treatment after being diagnosed with their first superficial cancer. Patients who underwent *H. pylori* eradication had a 0% four-year incidence of recurrence of superficial gastric cancer compared to a 9% incidence in those who did not.^[37]

But how does *H. pylori* affect the gastric epithelium if it's a major contributor to the occurrence of gastric cancer?

H. PYLORI IS A CARCINOGEN

A number of factors can contribute to gastric cancer. The type of bacterial strain, diet, and particularly the genetic specificity of the host are all factors that affect the development of this cancer if the bacterium represents an important link in its genesis. The bacterium *H. pylori* was identified by Krienitz in 1907. There are numerous *Helicobacter* species, some of which are gastric and others which are enterohepatic. *H. pylori* is the only pathogenic member of the *Helicobacter* family for humans. In developed nations, the colonization rate is lower (20 to 25% in France). This high prevalence reflects both the poor level of hygiene and the potential involvement of contamination by water, food, and human-to-human contamination linked to childhood promiscuity.^[38] The transmission has a very strong familial component. Additionally important are elements connected to poor socioeconomic conditions.^[39]

It is possible that this bacterium, which is only present in the human stomach or on gastric metaplasia of the duodenum, is the root cause or a contributing factor in the development of inflammatory diseases of the stomach. However, it could also just be an opportunistic commensal that thrives preferentially on a damaged

mucosa. In any case, it serves as a superb indicator of the gastric diseases it is linked to. More than 50% of people worldwide have *H. pylori* infection. Age, socioeconomic status, and geographic location all affect its prevalence. Even though *H. pylori* is immunogenic, the immune response does not offer protection from the diseases it causes.

H. pylori first establishes itself in the stomach after being ingested, leading to a variety of gastritis conditions that do not all progress to cancer. The condition known as antral gastritis results from *H. pylori* remaining confined to the antrum as the stomach's acid secretion increases. The duodenum is exposed to a significant acid load and experiences significant inflammation as a result of the abnormally high acid secretion, which frequently leads to a duodenal ulcer. In contrast, *H. pylori* will colonize the entire stomach if the stomach's acid secretion decreases, leading to pangastritis, which is an inflammation of the body and the antrum. Afterward, gastric ulceration occurs in the stomach itself. Inflammation has a significant impact on the architecture of the stomach, which in some cases exhibits atrophy. Installation of gut-like metaplasia may result from persistent inflammation.^[12,40] The presence of *H. pylori* infection can be identified through various methods. These include conducting a histological examination of the gastric mucosa, culturing endoscopic biopsy samples, or utilizing less invasive techniques such as the urea breath test (Clotest). The urea breath test involves ingesting urea labeled with Carbon C13, which is then hydrolyzed by the urease enzyme produced by *H. pylori*. This process leads to the formation of $^{13}\text{CO}_2$. The test involves analyzing the expiratory content to measure the presence of $^{13}\text{CO}_2$. Additionally, serological tests can be used to detect specific circulating IgG antibodies, indicating the presence of *H. pylori* infection. More frequently, serodiagnostic tests are used to assess the prevalence of infection in various populations. Three independent epidemiological studies that were all published in 1991 provided the first substantial evidence for the link between *H. pylori* infection and gastric cancer. In comparison to subjects without *H. pylori* infection, all three studies found that subjects who tested positive for *H. pylori* more than two decades before being diagnosed with gastric cancer had higher odds of developing the disease.^[41–43] Repeated studies of this nature have established the importance of *H. pylori* as the most important risk factor for the emergence of gastric cancer.^[44] Animal models provided the second piece of evidence implicating *H. pylori* as a risk factor for gastric pathology. Rodents have been used to model and study how *H. pylori* infection leads to the development of gastritis and gastric cancer. Following *H. pylori* infection, which is similar to the infection that affects humans, Mongolian gerbils experience duodenitis, ulcers, and

gastric cancer.^[45,46] In response to infection by *H. pylori* (strain SS1) and *H. felis*, atrophic gastritis and metaplasia are seen in mice. Contrarily, during an *H. felis* infection, progression to dysplasia and gastric cancer has been shown.^[47–49] Although the molecular mechanisms are partially understood, several pathways connect inflammation to cancer. Cells and their ability to proliferate can be severely harmed by the oxidative stress that macrophages and leukocytes produce during inflammation. Apoptosis and the prodigious release of cytokines are important contributors to the emergence of cancer.^[50]

Although infection is difficult to acquire and *H. pylori*-induced gastritis in mice is less severe than in humans, infection of conventional mice with *H. pylori* overcomes many issues.^[51] The Mongolian gerbil is an animal in which different gastrointestinal disorders can appropriately manifest, including gastritis, ulcers, intestinal metaplasia, and gastric cancer. The Mongolian gerbil model^[52–53] was used in the first animal report of *H. pylori* causing progression from intestinal metaplasia (pre-malignant lesion) to superficial gastritis.

It was established a few years later, using the same animal model, that *H. pylori* colonization can result in the growth of gastric cancer.^[54] In a report published more recently, *H. pylori* challenges were linked to the development of gastric cancer in a mouse model of the disease that several researchers had created.^[55] The understanding of *H. pylori* and its connection to gastric cancer has significantly advanced thanks to this model. An in vivo experiment using a transgenic mouse model showed that epithelial hyperplasia, gastric polyps, and adenocarcinoma were all associated with CagA expression, which is expressed primarily in the stomach. Leukocytosis, leukemia, and B-cell lymphomas have all been linked to systemic CagA expression.^[56]

IN VITRO

The development of gastric cancer and the gastric microbiome may be related, according to a growing body of research. Different stages of the disease have been described as causing changes in the gastric microbiome. The interactions between the host immune system, the environment, and the gastric microbiota are probably to blame for this. The homB gene was discovered to be responsible in 2021 by Keikha and Karbalaie for the progression from primary infection to serious complications, particularly peptic ulcer in Western countries and gastric cancer in Asian countries.

“Our work offers the first proof that the epithelial-mesenchymal transition induced by the bacterium results in the emergence of cancer stem cells that may cause

gastric cancers. And even though it is undoubtedly not the only mechanism responsible for the development of these cancers, it demonstrates how crucial the eradication of this bacterium” according to Emilie Bessè de. She claims that not all *H. pylori* strains produce the CagA protein: “In Asia, where the incidence of gastric cancer is very high, this protein is present in more than 90% of strains. However, CaA is only present in 50%–60% of the strains in France, where the prevalence of these cancers is more moderate. *In vitro*, using cellular models, the researchers investigated this lead. With or without the gene encoding the CagA protein, they infected gastric epithelial cells with *H. pylori* strains. After that, they looked at the expression of markers unique to cancer stem cells, mesenchymal cells, and epithelial cells. The scientists also investigated the functional characteristics of cells infected with bacteria carrying the *cagA* gene. In comparison to uninfected cells, it then seemed that these cells have greater invasive and migratory properties. Moreover, when transplanted into mice with impaired immunity, infected cells have the capacity to grow tumors. These traits describe cancer stem cells in general. *H. pylori*’s CagA gene may be oncogenic.

The chronic infection of the gastric mucosa caused by *H. pylori* results in a long-lasting inflammatory condition. T4SS is necessary for the induction of early and severe inflammation of the corpus, which is characterized by an increase in the expression of pro-inflammatory cytokines as well as histological changes with the occurrence of gastric atrophy and intestinal metaplasia. Cag PAI induces the synthesis by gastric epithelial cells of IL8, a potent pro-inflammatory cytokine. Numerous studies conducted in the West have linked bacterial infections with functional cag PAIs to peptic ulcers or CG.^[44]

The frequency of mutations in gastric epithelial cells increases with chronic *H. pylori* infection. Uncertainty surrounds the mechanism by which *H. pylori* infection causes gastric epithelial cells to mutate. 30 clinical isolates of *H. pylori* from 15 patients with gastric cancer and 15 patients with other gastroduodenal diseases were investigated by Kaneko *et al.*^[56] Using *Salmonella typhimurium* TA100, the Ames test was performed on 30 *H. pylori* strains to determine which ones were mutagenic. The findings show that none of the *H. pylori* strains tested were mutagenic, but the variation in Ames ratios between strains could be an indication of genotoxicity.^[57]

Arimoto-Kobayashi^[57] hypothesized in 20015 that *H. pylori* components might be connected to the mutagenic response brought on by DNA alkylation and could be identified using the Ames test using a more sensitive strain to alkylating agents. Their research showed that an extract of *H. pylori* was mutagenic in the Ames test with

Salmonella typhimurium YG7108, which has a defect in O 6-methylguanine DNA repair. They proposed that the mutagenic element is a small molecule that is absorbed into dialysis-resistant proteins in the *H. pylori* extract. The alkylative mutagenic component of chronically infected *H. pylori* in the stomach may be the cause of *H. pylori*-associated gastric carcinogenesis when it is continuously and chronically exposed to gastric epithelial cells.^[58]

In 1997, Hagman *et al.* investigated the mutagenicity of neutrophils exposed to two different strains of *H. pylori* for at least two hours, as well as the mutagenicity of sterile human gallbladder bile added alone to neutrophils or in combination with neutrophils and *H. pylori*. They discovered that the *H. pylori* and bile challenge to neutrophils causes mutagenicity. Reactive oxygen metabolites alone don’t seem to be the cause. According to reports, inflammation brought on by *Helicobacter* infection has a significant capacity to trigger DNA methylation. The presence of oxidatively damaged DNA in the gastric mucosa suggests an association with gastric carcinogenesis, and inflammation-related host factors linked to *H. pylori* infection have been reported to increase production of reactive oxygen species.^[59,26]

Papa and his team demonstrated in 2002^[60] that *H. pylori* infection caused by a CagA+ strain is connected to the highest production of reactive oxygen species, which equates to severe oxidative DNA damage. This series of events might lend credence to the idea that gastric carcinoma develops from chronic gastritis as a result of oxygen free radical-mediated damage caused by cytotoxic strains of the *H. pylori* bacteria. In 2015, Arimoto-Kobayashi and his team reported that an *H. pylori* extract was mutagenic in the Ames test with *Salmonella typhimurium* YG7108, which is deficient in DNA repair of O 6-methylguanine. It’s possible that the *H. pylori* extract contains methylating or alkylating agents that could cause DNA to become 6-alkylguanine. According to their hypothesis, the mutagenic substance is a small molecule that is absorbed by dialysis-resistant *H. pylori* extract proteins. A contributing factor to the development of *H. pylori*-associated gastric cancer may be the ongoing and chronic exposure of gastric epithelial cells to the alkylative mutagenic component of chronically infected *H. pylori* in the stomach.

The CagA oncoprotein and gastric cancer share an extensive and complex panel of virulence factors and effectors that are connected to the bacterium’s capacity to colonize and survive in its host while also causing harm to the gastric mucosa. The lesion can develop when *H. pylori* secretes the oncoprotein CagA, which, in combination with host factors such as pro-inflammatory cytokines, controls the inflammatory response.^[61] TFSS is used by *H. pylori* cagA-positive strains to start CagA

delivery into the cytoplasm of the host cells. The interaction of CagL, a component protein of SST4, with integrin 5β1 is crucial for the translocation of CagA,^[29] which initiates the formation of a conduit connecting the bacterium and the host cytoplasm to deliver CagA.

A significant mucosal and systemic immune response brought on by *H. pylori* infection results in the production of antibodies.^[61] But neither the humoral nor the oxidative response can put an end to the infection; instead, severe inflammation causes damage to the stomach's lining and may result in atrophic gastritis. The beginning of an inflammatory response in the gastric mucosa is the primary physiological event in *H. pylori* infection. The inflammatory cytokines IL-1 and TNF-α are especially stimulated by *H. pylori*, its products, and many non-microbial agents. Inflammatory cytokines are soluble peptide molecules that mediate communication between immune and neuroendocrine systems as well as between immunocompetent and hematopoietic cells. Once *H. pylori* has infected the gastric mucosa, it activates neutrophils and mononuclear cells, which then infiltrate the mucosa and release a number of inflammatory cytokines.^[62] By attaching to particular receptors on target cells,^[63] these cytokines produce their biological effects.

By avoiding lysosomal fusion, *H. pylori* can also evade bacterial phagocytosis, in which PNNs and macrophages take part.^[64] As a result, it has been demonstrated that *H. pylori* bacteria can survive intracellularly in macrophages by interfering with lysosomal proteins.^[65] In one study, Allen and his team demonstrated delayed bacterial uptake into macrophages, followed by the formation of megasomes as a result of phagosome fusion. These megasomes shield intracellular bacteria from effective eradication and may even help an infection persist.^[64,66] Finally, more recently, the Zhao team showed that activated myeloid suppressor cells and IL-17-producing γδ T cells were present in the gastric tumor tissue, suggesting a role for these two cell populations in the development of LGM,^[67] both in patients with LGM and in a mouse model of LGM induced by *H. felis* infection.

Activated cancer-associated fibroblasts (CAF), the majority of the cells surrounding the gastric cancer that are influenced by CagA, produce a molecular microenvironment that encourages tumorigenesis and cancer invasion.^[68,69] *H. pylori* CagA-positif can induce activation and differentiation of gastric fibroblasts, mediated by the transcription factors NFκB and STAT3 signaling leading to rapid expression of the Snail1 protein, which can finally activate the secretome responsible for the inflammatory and fibroblast-inducing microenvironment that serves in gastric cancer development.^[70]

Bacterial strains that are positive for both proteins

(CagA and VacA), which have complementary functions, have higher virulence and are more capable of determining the histopathological actions that take place in the oncogenesis pathway. As a result, VacA and CagA alter adhesion, cytoskeletal organization, cell mobility and permeability, as well as mitochondrial and lysosomal activities.^[71]

CagA and VacA have an antagonistic effect on one another: By simulating Bcl2's antiapoptotic properties, CagA prevents VacA from exerting its toxic effects. CagA's phosphorylated form prevents VacA from moving into intracellular compartments, while its non-phosphorylated form blocks VacA from doing so. This antagonistic relationship is also seen in the morphological changes that *H. pylori* causes in epithelial cells: VacA+/CagA- mutant strains worsen the vacuolation of host cells, while CagA+/VacA- mutants increase the length of the cells. The ERK pathway is not activated by CagA when VacA is present.^[72]

Individual clinical outcomes are clearly influenced by host genetic polymorphisms, which have a direct impact on inter-individual variations in cytokine production rates that are both anti- and pro-inflammatory. Humans develop a potent cellular and humoral immune response to *H. pylori*. The humoral reaction does not appear to be capable of curing the infection.^[73] By infiltrating macrophages, neutrophils, regulatory T cells, and natural killer cells, *H. pylori* infection has a significant impact on the gastric microenvironment.^[11,74] Inflammatory mediators such as cytokines, chemokines, and metalloproteinases that are released by gastric and infiltrating cells promote the EMT process in gastric cells; transforming growth factor-β (TGF-β) is probably one of the most relevant EMT inducers.^[12]

Therefore, chronic inflammation may play a significant role in the development of EMT and carcinogenesis.^[75]

Small, secreted proteins called cytokines are linked to the preservation of immune homeostasis and have also been linked to the pathogenesis of a number of autoimmune and inflammatory diseases. The treatment of such pathologies has been transformed by biological agents that block cytokines or their receptors. To treat inflammatory bowel disease that targets cytokines, cytokine receptors, and adhesion molecules, many difficulties have recently been identified.^[76]

The immune response, though, might not be sufficient to get rid of the infection. In fact, a study done on mice lacking the anti-inflammatory cytokine IL-10 showed that the innate immune system can stop *H. pylori* infection.^[77] Therefore, the long-term persistence of this infection is actually caused by the immune system being modulated by *H. pylori*. The immune response to *H.*

pylori, which involves a delicate balance between innate immunity actors and dependent adaptive responses TH1 and TH17, significantly affects how the infection develops.

Numerous immunomodulation techniques are used by *H. pylori*. On the other hand, excessively intense inflammation must be avoided by the host as it can help control infection but is harmful to the gastric mucosa. For *H. pylori*, the secret to an infection that has persisted for years without treatment is its capacity to trigger an anti-inflammatory response in order to avoid being eliminated by its host. Everything is therefore a matter of balance, and the relatively harmonious coexistence between *H. pylori* and its host is disrupted when one of the responses takes over.

A study was conducted to determine how the CagA gene affects the expression of the cytokine messenger RNA (mRNA gastroduodenal diseases associated with *H. pylori* infection) in the gastric mucosa. Inducing IL-8 mRNA expression by CagA-positive strains suggests that IL-8 may be crucial in the pathogenesis of gastroduodenal disease brought on by *H. pylori* infection.^[78] It has a type IV secretion system that can introduce molecules into the epithelial cell, including the CagA protein. This protein will interfere with actin polymerization, reorganize the cytoskeleton, and undoubtedly promote apoptosis.

Gastric adenocarcinomas are caused by chronic atrophic gastritis, autoimmune atrophic gastritis (Biermer's disease), and inflammation of the gastric mucosa followed by destruction of the superficial layer by *Helicobacter pylori*. one percent of *H. pylori* patients develop adenocarcinoma. Silently, it has evolved.

TUMOR NECROSIS FACTOR

TNF- α inducing protein (Tip protein α), which binds to cell surface nucleolin and triggers carcinogenic changes, is produced in large quantities by *H. pylori* strains.^[79,80] It is one of the potential indicators of *H. pylori*'s virulence. Tip α promotes EMT progression by interacting with the nucleolin receptor to cause mesenchymal changes in cells.^[81] *H. pylori* isolated from gastric cancer patients can secrete significant amounts of Tip. TGF- α , hepatocyte growth factor (HGF α), tumor necrosis factor α (TNF- α), and hypoxia-inducible factor 1- α (HIF1 α) all support EMT. Tip α , CagA, and VacA all contribute to mucosal damage, which is a major factor in gastric carcinogenesis.^[82]

According to research, Tip α promotes the formation of filopodia in gastric cancer cell lines, indicating invasive morphological changes and decreased Young's modulus

of gastric cancer cells, which indicates that cell stiffness is reduced and cell motility is increased and phosphorylates various oncoproteins; it also enhances the formation of filopodia, morphological and conformational changes within cells, and vimentin expression via MEK-ERK phosphorylation, confirming its role in these processes.^[83,84]

PROGRAMMED CELL DEATH PROTEIN

Apoptosis, also known as programmed cell death, which literally translates to "falling off" like leaves, is a cellular process that gets rid of unhealthy, unwanted, or dangerous cells. Rarely, if ever, does *H. pylori* invade the stomach lining. Although *H. pylori* can colonize stomach crypts, it never spreads. The primary cause of the host response is bacterial attachment to epithelial cells. Class II MHC is found on the surface of gastric epithelial cells, and *H. pylori* can bind it to cause their apoptosis.^[85]

A tumor suppressor called programmed cell death protein 4 (PDCD4) prevents tumor invasion, metastasis, or the induction of apoptosis in cells. It binds to eukaryotic initiation factor 4A (eIF4A) or eukaryotic translation initiation factor 4G (eIF4G), which prevents translation, and is found in the nucleus of proliferating cells. By controlling the expression of mitogen-activated protein 4 kinase 1 or urokinase plasminogen activator receptor, PDCD4 regulates the inhibition of cancer invasion. Along with connecting with the DNA binding domain of the TWIST1 gene, it is also in charge of suppressing the expression of the Y-box binding protein.^[86]

One of the elements contributing to the induction of carcinogenesis is PDCD4.^[87] Yu and his team^[88] demonstrated that in gastric cancer tissue, downregulating PDCD4 expression results in lower levels of E-cadherin, higher levels of TWIST1, and lower levels of vimentin. CagA-positive *H. pylori* strain infection caused the downregulation of PDCD4 and subsequent alterations in mesenchymal epithelial markers. EMT was reduced by overexpression of PDCD4. In gastric cancer, overexpression of microRNA-21 affects the expression of the tumor suppressors phosphatase and tensin homolog (PTEN) and PDCD4, changing the molecular pathways involved in cell growth, invasion, migration, and apoptosis.^[89]

CONCLUSION

It is still debatable whether *H. pylori* infection causes gastric carcinogenesis. The advancement of sequencing technology has increased our understanding of the human gastric microbiome, which is now known to be essential for preserving homeostasis while modifications

to the make-up of the microbial community may encourage the emergence of gastric diseases. The gastric microbiome's carcinogenic effects have drawn more attention recently.

The development of gastric cancer is also aided by bacteria other than *H. pylori*. In a high-risk area of China, a prospective randomized controlled trial showed that over the course of 7.5 years, patients receiving *H. pylori* eradication therapy compared to placebo experienced a similar incidence of gastric cancer.^[90] According to a recent case-control study on the microbiome profiles of the stomach conducted in Korea, patients with gastric cancer had higher concentrations of *P. acnes* and *P. copri* than control subjects, indicating that the presence of these species raises the risk of gastric cancer.^[91]

There is mounting evidence linking the development of gastric cancer and the gastric microbiome. At various disease stages, the gastric microbiome has undergone described changes. This is probably brought on by interactions between the host immune system, the environment, and the gastric microbiota. Despite numerous studies on the *H. pylori*'s carcinogenic mechanisms in recent decades regarding the precise part of non-*H. pylori* in the emergence of gastric cancer. Currently, only a few studies have concentrated on the potential carcinogenic roles of non-*H. pylori* and their metabolites, including induction of inflammatory response, modulation of immune response, induction of DNA damage, and promotion of EMT. As a result, more research is needed to elucidate the detailed carcinogenic mechanisms of the gastric microbiome and provide new insights for the diagnosis, prevention, and treatment of gastric cancer.

However, it is difficult to propose eradicating this bacterium on a population scale in the hope of curing this cancer. More research is needed to identify infected individuals at risk (characterization of the bacterial strains involved in gastric cancer and predictive susceptibility markers) who should benefit from an eradication cure to prevent the occurrence of gastric cancer.

DECLARATIONS

Author contributions

Asma Boudouaia-Ouali is the lead author of this review under the supervision of Professor Dali-Sahi.

Conflicts of interest

There is no conflict of interest among the authors.

Data sharing statement

No additional data is available.

REFERENCES

1. Atsushi T, Tamano H. New insight into Parkinson's disease pathogenesis from reactive oxygen species-mediated extracellular Zn(2+) influx. *J Trace Elem Med Biol* 2020;61:126545.
2. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol* 2019;14:89-103.
3. Megraud F. When and how does *Helicobacter pylori* infection occur? *Gastroenterol Clin Biol*. mars 2003;27(3 Pt 2):3749.
4. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, *et al*. Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. *Gastroenterology* 2017;153:420-429.
5. Schistosomes, liver flukes and *Helicobacter pylori*. *IARC Monogr Eval Carcinog Risks Hum*. 1994;61:1-241.
6. Courteous S. Study of metformin effects and involvement of the mTOR signaling pathway in *Helicobacter pylori* infection and gastric carcinogenesis. University of Bordeaux, 2017.
7. Zabala Torres B, Lucero Y, Lagomarcino AJ, Orellana-Manzano A, George S, Torres JP, *et al*. Review: Prevalence and dynamics of *Helicobacter pylori* infection during childhood. *Helicobacter* 2017;22.
8. Umeda M, Kobayashi H, Takeuchi Y, Hayashi J, Morotome-Hayashi Y, Yano K, *et al*. High prevalence of *Helicobacter pylori* detected by PCR in the oral cavities of periodontitis patients. *J Periodontol* 2003;74:129-134.
9. Chen T, You Y, Jiang H, Wang ZZ. Epithelial-mesenchymal transition (EMT): A biological process in the development, stem cell differentiation, and tumorigenesis. *J Cell Physiol* 2017;232:3261-3272.
10. Bessède E, Staedel C, Acuña Amador LA, Nguyen PH, Chambonnier L, Hatakeyama M, *et al*. *Helicobacter pylori* generates cells with cancer stem cell properties via epithelial-mesenchymal transition-like changes. *Oncogene* 2014;33:4123-4131.
11. Ma HY, Liu XZ, Liang CM. Inflammatory microenvironment contributes to epithelial-mesenchymal transition in gastric cancer. *World J Gastroenterol* 2016;22:6619-6628.
12. Singh S, Gill AAS, Nlooto M, Karpoomath R. Prostate cancer biomarkers detection using nanoparticles based electrochemical biosensors. *Biosens Bioelectron* 2019;137:213-221.
13. Seyda T, Derya C, Füsün A, Meliha K. The relationship of *Helicobacter pylori* positivity with age, sex, and ABO/Rhesus blood groups in patients with gastrointestinal complaints in Turkey. *Helicobacter* 2007;12:244-250.
14. Laszewicz W, Iwańczak F, Iwańczak B; Task Force of the Polish Society of Gastroenterology; Task Force of the Polish Society of Gastroenterology. Seroprevalence of *Helicobacter pylori* infection in Polish children and adults depending on socioeconomic status and living conditions. *Adv Med Sci* 2014;59:147-150.
15. Alberts J, Löwe B, Glahn MA, Petrie K, Laferton J, Nestoriuc Y, Shedden-Mora M. Development of the generic, multidimensional Treatment Expectation Questionnaire (TEX-Q) through systematic literature review, expert surveys and qualitative interviews. *BMJ Open* 2020; 10:e036169.
16. Banatvala N, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and *Helicobacter pylori*. *J Infect Dis* 1993;168:219-221.
17. Testerman TL, Morris J. Beyond the stomach: an updated view of *Helicobacter pylori* pathogenesis, diagnosis, and treatment. *World J Gastroenterol* 2014;20:12781-12808.
18. Brenner H, Arndt V, Stürmer T, Stegmaier C, Ziegler H, Dhon G. Individual and joint contribution of family history and *Helicobacter pylori* infection to the risk of gastric carcinoma. *Cancer*. 2000 15;88:274-9.
19. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH,

- Young H, *et al.* Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; 404:398-402.
20. Jiang Q, Hiratsuka K, Taylor DE. Variability of gene order in different *Helicobacter pylori* strains contributes to genome diversity. *Mol Microbiol* 1996; 20:833-842.
21. Linz B, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, *et al.* An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature* 2007;445:915-918.
22. Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, *et al.* The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* 1997;388:539-547.
23. Janvier B, Grignon B, Audibert C, Pezenne L, Fauchère JL. Phenotypic changes of *Helicobacter pylori* components during an experimental infection in mice. *FEMS Immunol Med Microbiol* 1999;24:27-33.
24. Arnion H. Study of small RNAs in a bacterium: *Helicobacter pylori*. [dissertation]. Versailles: Université de Versailles/St-Quentin. 2011 Nov 25. 28 p.
25. Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, *et al.* Traces of human migrations in *Helicobacter pylori* populations. *Science*. 2003;299:1582-5.
26. Bommelaer G, Stef A. Gastrointestinal ulcer before and after *Helicobacter pylori*. *Gastroenterol Clin Biol* 2009;33:626-634.
27. Akopyants NS, Clifton SW, Kersulyte D, Crabtree JE, Youree BE, Reece CA, *et al.* Analyses of the *cag* pathogenicity island of *Helicobacter pylori*. *Mol Microbiol* 1998;28:37-53.
28. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, *et al.* *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci USA* 1996;93:14648-14653.
29. Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, Misselwitz R, Berger J, Sewald N, König W, Backert S. *Helicobacter* exploits integrin for type IV secretion and kinase activation. *Nature* 2007;449:862-866.
30. Asahi M, Azuma T, Ito S, Ito Y, Suto H, Nagai Y, Tsubokawa M, Tohyama Y, Maeda S, Omata M, Suzuki T, Sasakawa C. *Helicobacter pylori* CagA protein can be tyrosine phosphorylated in gastric epithelial cells. *J Exp Med* 2000;191:593-602.
31. Odenbreit S, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science* 2000;287:1497-1500.
32. Stein M, Rappuoli R, Covacci A. Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after *cag*-driven host cell translocation. *Proc Natl Acad Sci USA* 2000;97:1263-1268.
33. Backert S, Ziska E, Brinkmann V, Zimny-Arndt U, Fauconnier A, Jungblut PR, Naumann M, Meyer TF. Translocation of the *Helicobacter pylori* CagA protein in gastric epithelial cells by a type IV secretion apparatus. *Cell Microbiol* 2000;2:155-164.
34. Murata-Kamiya N, Kikuchi K, Hayashi T, Higashi H, Hatakeyama M. *Helicobacter pylori* exploits host membrane phosphatidylserine for delivery, localization, and pathophysiological action of the CagA oncoprotein. *Cell Host Microbe* 2010;7:399-411.
35. Higashi H, Yokoyama K, Fujii Y, Ren S, Yuasa H, Saadat I, Murata-Kamiya N, Azuma T, Hatakeyama M. EPIYA motif is a membrane-targeting signal of *Helicobacter pylori* virulence factor CagA in mammalian cells. *J Biol Chem* 2005;280:23130-23137.
36. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001;345:784-789.
37. de Beauregard MB, Lamarque D. What are the environmental factors that promote gastric cancer? *Hepato-Gastro Oncol Dig*. 2011;18:601-606.
38. Brown LM. *Helicobacter pylori*: epidemiology and routes of transmission. *Epidemiol Rev* 2000;22:283-297.
39. Correa P. Human gastric carcinogenesis: a multistep and multifunctional process. First American cancer society award lecture on cancer epidemiology and prevention. *Cancer Res*. 1992;52:6735-40.
40. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 1991;302:1302-1305.
41. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 1991;325:1132-1136.
42. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelstein JH, Orentreich N, Sibley RK. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 1991;325:1127-1131.
43. Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between *cagA* seropositivity and gastric cancer. *Gastroenterology* 2003;125:1636-1644.
44. Honda S, Fujioka T, Tokieda M, Satoh R, Nishizono A, Nasu M. Development of *Helicobacter pylori*-induced gastric carcinoma in mongolian gerbils. *Cancer Res*. 1998;58:4255-4259.
45. Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M. *Helicobacter pylori* infection induces gastric cancer in mongolian gerbils. *Gastroenterology* 1998;115:642-648.
46. Wang TC, Goldenring JR, Dangler C, Ito S, Mueller A, Jeon WK, *et al.* Mice lacking secretory phospholipase A2 show altered apoptosis and differentiation with *Helicobacter felis* infection. *Gastroenterology* 1998;114:675-689.
47. Fox JG. The non-H pylori helicobacters: their expanding role in gastrointestinal and systemic diseases. *Gut* 2002;50:273-283.
48. Cai X, Carlson J, Stoicov C, Li H, Wang TC, Houghton J. *Helicobacter felis* eradication restores normal architecture and inhibits gastric cancer progression in C57BL/6 mice. *Gastroenterology* 2005;128:1937-1952.
49. Allavena P, Garlanda C, Borrello MG, Sica A, Mantovani A. Pathways connecting inflammation and cancer. *Curr Opin Genet Dev* 2008;18:3-10.
50. Marchetti M, Aricò B, Burrone D, Figura N, Rappuoli R, Ghiara P. Development of a mouse model of *Helicobacter pylori* infection that mimics human disease. *Science* 1995;267:1655-1658.
51. Ogura K, Maeda S, Nakao M, Watanabe T, Tada M, Kyutoku T, Yoshida H, Shiratori Y, Omata M. Virulence factors of *Helicobacter pylori* responsible for gastric diseases in Mongolian gerbil. *J Exp Med* 2000;192:1601-1610.
52. Hirayama F, Takagi S, Yokoyama Y, Iwao E, Ikeda Y. Establishment of gastric *Helicobacter pylori* infection in Mongolian gerbils. *J Gastroenterol* 1996;31 Suppl 9:24-28.
53. Hirayama F, Takagi S, Iwao E, Yokoyama Y, Haga K, Hanada S. Development of poorly differentiated adenocarcinoma and carcinoid due to long-term *Helicobacter pylori* colonization in Mongolian gerbils. *J Gastroenterol* 1999;34:450-454.
54. Poh AR, O'Donoghue RJ, Ernst M, Putoczki TL. Mouse models for gastric cancer: Matching models to biological questions. *J Gastroenterol Hepatol* 2016;31:1257-1272.
55. Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M, Matsui A, *et al.* Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. *Proc Natl Acad Sci U S A* 2008;105:1003-1008.
56. Kaneko T, Kawakami Y, Akaimatsu T, Kiyosawa K, Katsuyama T. Mutagenicity of *Helicobacter pylori* in the Ames test using *Salmonella typhimurium* TA100. *J Int Med Res* 2000;28:222-228.
57. Arimoto-Kobayashi S, Ohta K, Yuhara Y, Ayabe Y, Negishi T, Okamoto K, Nakajima Y, Ishikawa T, Oguma K, Otsuka T. Mutagenicity and clastogenicity of extracts of *Helicobacter pylori* detected by the Ames test and in the micronucleus test using human lymphoblastoid cells. *Mutagenesis* 2015;30:537-544.
58. Touati E, Michel V, Thiberge JM, Wuscher N, Huerre M, Labigne A. Chronic *Helicobacter pylori* infections induce gastric mutations in mice.

- Gastroenterology 2003;124:1408-1419.
59. Izzotti A, De Flora S, Cartiglia C, Are BM, Longobardi M, Camoirano A, Mura I, Dore MP, Scanu AM, Rocca PC, Maida A, Piana A. Interplay between *Helicobacter pylori* and host gene polymorphisms in inducing oxidative DNA damage in the gastric mucosa. *Carcinogenesis* 2007;28:892-898.
 60. Papa A, Danese S, Sgambato A, Ardito R, Zannoni G, Rinelli A, Vecchio FM, Gentiloni-Silveri N, Cittadini A, Gasbarrini G, Gasbarrini A. Role of *Helicobacter pylori* CagA+ infection in determining oxidative DNA damage in gastric mucosa. *Scand J Gastroenterol* 2002;37:409-413.
 61. Perez-Perez GI, Dworkin BM, Chodos JE, Blaser MJ. *Campylobacter pylori* antibodies in humans. *Ann Intern Med* 1988;109:11-17.
 62. Fridman WH, Tartour E. Cytokines and cell regulation. *Mol Aspects Med* 1997;18:3-90.
 63. Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY, *et al.* Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1 β production in *Helicobacter pylori* infection. *Gastroenterology* 2002;123:1793-1803.
 64. Allen A, Flemström G. Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. *Am J Physiol Cell Physiol* 2005;288:C1-19.
 65. Ramarao N, Meyer TF. *Helicobacter pylori* resists phagocytosis by macrophages: quantitative assessment by confocal microscopy and fluorescence-activated cell sorting. *Infect Immun* 2001;69:2604-2611.
 66. Schwartz JT, Allen LA. Role of urease in megasome formation and *Helicobacter pylori* survival in macrophages. *J Leukoc Biol* 2006;79:1214-1225.
 67. Zhao Y, Li Y, Hu J, Wang X, Ren M, Lu G, Lu X, Zhang D, He S. The Effect of *Helicobacter pylori* Eradication in Patients with Gastroesophageal Reflux Disease: A Meta-Analysis of Randomized Controlled Studies. *Dig Dis* 2020;38:261-268.
 68. Krzysiek-Maczka G, Targosz A, Ptak-Belowska A, Korbut E, Szczyrk U, Strzalka M, *et al.* Molecular alterations in fibroblasts exposed to *Helicobacter pylori*: a missing link in bacterial inflammation progressing into gastric carcinogenesis? *J Physiol Pharmacol.* 2013;64:77-87.
 69. Lim H, Moon A. Inflammatory fibroblasts in cancer. *Arch Pharm Res* 2016;39:1021-1031.
 70. Krzysiek-Maczka G, Targosz A, Szczyrk U, Strzalka M, Brzozowski T, Ptak-Belowska A. Involvement of epithelial-mesenchymal transition-inducing transcription factors in the mechanism of *Helicobacter pylori*-induced fibroblasts activation. *J Physiol Pharmacol.* 2019;70:72.
 71. Ly A. *Helicobacter pylori*'s infections and oncogenesis. *J Afr Cancer.* 2010;2:213-218.
 72. Tegtmeyer N, Zabler D, Schmidt D, Hartig R, Brandt S, Backert S. Importance of EGF receptor, HER2/Neu and Erk1/2 kinase signalling for host cell elongation and scattering induced by the *Helicobacter pylori* CagA protein: antagonistic effects of the vacuolating cytotoxin VacA. *Cell Microbiol* 2009;11:488-505.
 73. Ferrero RL, Thiberge JM, Huerre M, Labigne A. Immune responses of specific-pathogen-free mice to chronic *Helicobacter pylori* (strain SS1) infection. *Infect Immun* 1998;66:1349-1355.
 74. Baj J, Korona-Glowniak I, Forma A, Maani A, Sitarz E, Rahnama-Hezavah M, *et al.* Mechanisms of the Epithelial-Mesenchymal Transition and Tumor Microenvironment in *Helicobacter pylori*-Induced Gastric Cancer. *Cells* 2020;9.
 75. Soundararajan R, Fradette JJ, Konen JM, Moulder S, Zhang X, Gibbons DL, *et al.* Targeting the Interplay between Epithelial-to-Mesenchymal-Transition and the Immune System for Effective Immunotherapy. *Cancers (Basel)* 2019;11.
 76. Nakamura K, Honda K, Mizutani T, Akiho H, Harada N. Novel strategies for the treatment of inflammatory bowel disease: Selective inhibition of cytokines and adhesion molecules. *World J Gastroenterol* 2006;12:4628-4635.
 77. Chen CN, Sung CT, Lin MT, Lee PH, Chang KJ. Clinicopathologic association of cyclooxygenase 1 and cyclooxygenase 2 expression in gastric adenocarcinoma. *Ann Surg* 2001;233:183-188.
 78. Yamaoka Y, Kita M, Kodama T, Sawai N, Imanishi J. *Helicobacter pylori* cagA gene and expression of cytokine messenger RNA in gastric mucosa. *Gastroenterology* 1996;110:1744-1752.
 79. Tsuge H, Tsurumura T, Utsunomiya H, Kise D, Kuzuhara T, Watanabe T, Fujiki H, Suganuma M. Structural basis for the *Helicobacter pylori*-carcinogenic TNF- α -inducing protein. *Biochem Biophys Res Commun* 2009;388:193-198.
 80. Ashrafi H, Siraji MI, Showva NN, Hossain MM, Hossain T, Hasan MA, Shohael AM, Shawan MMAK. Structure to function analysis with antigenic characterization of a hypothetical protein,HPAG1_0576 from *Helicobacter pylori* HPAG1. *Bioinformation* 2019;15:456-466.
 81. Huber MA, Azoitei N, Baumann B, Grünert S, Sommer A, Pehamberger H, Kraut N, Beug H, Wirth T. NF- κ B is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. *J Clin Invest* 2004;114:569-581.
 82. Fujiki H, Sueoka E, Watanabe T, Suganuma M. The concept of the okadaic acid class of tumor promoters is revived in endogenous protein inhibitors of protein phosphatase 2A, SET and CIP2A, in human cancers. *J Cancer Res Clin Oncol* 2018;144:2339-2349.
 83. Suganuma M, Kuzuhara T, Yamaguchi K, Fujiki H. Carcinogenic role of tumor necrosis factor- α inducing protein of *Helicobacter pylori* in human stomach. *J Biochem Mol Biol* 2006;39:1-8.
 84. Fujiki H, Sueoka E, Suganuma M. Tumor promoters: from chemicals to inflammatory proteins. *J Cancer Res Clin Oncol* 2013;139:1603-1614.
 85. Fan X, Gunasena H, Cheng Z, Espejo R, Crowe SE, Ernst PB, Reyes VE. *Helicobacter pylori* urease binds to class II MHC on gastric epithelial cells and induces their apoptosis. *J Immunol* 2000;165:1918-1924.
 86. Shiota M, Izumi H, Tanimoto A, Takahashi M, Miyamoto N, Kashiwagi E, Kidani A, Hirano G, Masubuchi D, Fukunaka Y, Yasuniwa Y, Naito S, Nishizawa S, Sasaguri Y, Kohno K. Programmed cell death protein 4 down-regulates Y-box binding protein-1 expression via a direct interaction with Twist1 to suppress cancer cell growth. *Cancer Res* 2009;69:3148-3156.
 87. Jo SD, Ku SH, Won YY, Kim SH, Kwon IC. Targeted Nanotheranostics for Future Personalized Medicine: Recent Progress in Cancer Therapy. *Theranostics* 2016;6:1362-1377.
 88. Yu H, Zeng J, Liang X, Wang W, Zhou Y, Sun Y, Liu S, Li W, Chen C, Jia J. *Helicobacter pylori* promotes epithelial-mesenchymal transition in gastric cancer by downregulating programmed cell death protein 4 (PDCD4). *PLoS One* 2014;9:e105306.
 89. Li L, Gu H, Zhang G. Association between recurrent aphthous stomatitis and *Helicobacter pylori* infection: a meta-analysis. *Clin Oral Investig* 2014;18:1553-1560.
 90. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS; China Gastric Cancer Study Group. *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004;291:187-194.
 91. Gunathilake MN, Lee J, Choi IJ, Kim YI, Ahn Y, Park C, Kim J. Association between the relative abundance of gastric microbiota and the risk of gastric cancer: a case-control study. *Sci Rep* 2019;9:13589.