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Helicobacter pylori infection: Host immune response, implications on gene expression and microRNAs

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Abstract

Helicobacter pylori (*H. pylori*) infection is the most common bacterial infection worldwide. Persistent infection of the gastric mucosa leads to inflammatory processes and may remain silent for decades or progress causing more severe diseases, such as gastric adenocarcinoma. The clinical consequences of *H. pylori* infection are determined by multiple factors, including host genetic predisposition, gene regulation, environmental factors and heterogeneity of *H. pylori* virulence factors. After decades of studies of this successful relationship between pathogen and human host, various mechanisms have been elucidated. In this review, we have made an introduction on *H. pylori* infection and its virulence factors, and focused mainly on modulation of host immune response triggered by bacteria, changes in the pattern of gene expression in *H. pylori*-infected gastric mucosa, with activation of gene transcription involved in defense mechanisms, inflammatory and immunological response, cell proliferation and apoptosis. We also highlighted the role of bacteria eradication on gene expression levels. In addition, we addressed the recent involvement of different microRNAs in precancerous lesions, gastric cancer, and inflammatory processes in-

duced by bacteria. New discoveries in this field may allow a better understanding of the role of major factors involved in the pathogenic mechanisms of *H. pylori*.

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Key words: *Helicobacter pylori*; Inflammation; Virulence factors; Immune response; Gastric lesions; Gastric cancer; Gene expression; MicroRNAs

Core tip: In this review, we focused some aspects of *Helicobacter pylori* (*H. pylori*) infection as bacterial virulence factor and mainly on modulation of host immune response and changes in the pattern of gene expression in *H. pylori*-infected gastric mucosa, with activation of gene transcription involved in inflammatory and immunological response, cell proliferation and apoptosis. We also highlighted the role of bacteria eradication for the normalization of gene expression levels. In addition, we addressed the recent involvement of different microRNAs in normal gastric mucosa, precancerous lesions, gastric cancer, and inflammatory processes induced by bacteria, showing deregulated expression.

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INTRODUCTION

Infection by *Helicobacter pylori* (*H. pylori*), a Gram-negative, microaerophilic, spiral-shaped bacteria that colonizes the gastric mucosa, is considered the most common bacterial infection worldwide. It is usually acquired during

childhood and may persist in the gastric environment throughout life if not treated^[1,2]. The persistent presence of *H. pylori* in the stomach can result in chronic gastritis and may remain silent for decades after infection, due to the synchronized balance between the pathogen and its host, or cause more severe diseases such as atrophic gastritis, peptic ulcer, mucosa-associated lymphoid tissue lymphoma or gastric adenocarcinoma^[2,3]. Therefore, *H. pylori* infection is considered the strongest factor associated with this neoplasm, mainly due to the inflammatory process triggered in the gastric mucosa, increasing the risk of gastric cancer over six-fold compared to individuals without this infection^[4,5]. Gastric cancer is considered the first one among the several cancer types associated with infection in the world, with almost 75% of cases being attributable to *H. pylori* infection^[6]. As a consequence of this association, *H. pylori* was classified in 1994 by the International Agency for Research on Cancer as a type I carcinogen^[7].

H. pylori infects over half of the world population, but there is variation in incidence among different geographic regions^[8]. Eighty-five percent of *H. pylori*-infected individuals remain lifelong asymptomatic, while only 1% of these individuals develop gastric cancer^[9] and 10% develop peptic ulcer^[10].

Indeed, the clinical consequences of infection by *H. pylori* are determined by multiple factors, including genetic predisposition of the host, especially regarding certain cytokine, and receptor gene polymorphisms^[11-15], gene regulation, environmental factors such as high dietary salt intake, and heterogeneity of *H. pylori* strains^[2,16,17].

In most cases, *H. pylori* infection can persist lifelong in its host in the absence of eradicating antibiotics^[11,18], because it is capable of adaptations to colonize the adverse environment of the stomach. *H. pylori* can survive in the gastric environment at a wide range of pHs, due to urease enzyme activity and the presence of flagella which facilitate the penetration into the mucus layer and reaching the gastric epithelium^[19]. Urease hydrolyzes urea to ammonia and carbon dioxide, neutralizing the pH, which allows the bacterial survival and proliferation^[19], circumventing host defenses such as the immune response^[20].

In general, it is noted that colonization by *H. pylori* causes a strong systemic immune response, creating a chronically inflamed environment with reduced stomach acidity that favors the growth of other bacteria in the gastric environment, maintaining the inflammation and thereby reducing the level of vitamin C in the gastric juice. The inhibition of gastric acid secretion favors a change from antrum-predominant to corpus-predominant gastritis, initiating gastric atrophy and intestinal metaplasia, which characterize precancerous lesions^[21].

Furthermore, the bacterial virulence factors cytotoxin-associated gene A antigen (CagA) and vacuolating cytotoxin (VacA) play a pivotal role in *H. pylori*-induced pathogenesis, and others, such as IceA (induced by contact with epithelium), blood group antigen-binding adhesion (BabA), sialic acid-binding adhesion (SabA), duodenal ulcer-promoting gene (DupA) and outer inflammatory protein (OipA), also allow a successful colo-

nization of the mucosa^[22,23]. These bacteria populations are highly heterogeneous with respect to virulence factors VacA and CagA^[24], and several substantial pieces of evidence show that these genetic differences play an important role in the clinical outcome of the infection^[17,25].

The *cagA* gene produces one of the most important virulence factors of *H. pylori*, being located in a segment of DNA called the *cag* pathogenicity island (cagPAI) that contains, besides the *cagA* gene, genes which give rise to the bacterial type IV secretion system (T4SS-type-IV secretion system)^[26]. This system functions like a molecular syringe, injecting CagA, peptidoglycans and other factors into host epithelial cells^[27]. After its entry into the cell, CagA can be phosphorylated by tyrosine kinases and interact with cellular proteins, acting in the signal transduction pathways to the nucleus, changes in the cytoskeleton, disruption of cell-cell junctions^[28,29], stimulating the growth factor signaling, leading to changes in cell morphology and increased cell proliferation^[30], as well as anti-apoptotic responses^[29,31]. CagA is not found in all strains of Western *H. pylori* population^[32]. Its occurrence is associated with more severe inflammation of the gastric mucosa^[33,34], conferring a greater risk of developing stomach cancer^[32,35,36].

The second most studied virulence factor is the VacA, encoded by *vacA* gene that induces the formation of vacuoles in eukaryotic cells and stimulates apoptosis in epithelial cells^[37]. Unlike *cagA*, all *H. pylori* strains possess the *vacA* gene, although only about 50% of them express the VacA protein. The regions with the highest diversity are located at the 5' terminus signal (allele types s1a, s1b, s1c and s2), the mid-region (allele types m1 and m2) and the intermediate region (allele types i1 and i2)^[38]. This combination of sequence diversity in *vacA*, considering that each gene contains a single allele (signal, mid-region and intermediate region allele), causes variations in cytotoxic activity^[39], the s1m1 strain being highly toxigenic^[40]. Humans infected with *H. pylori*-VacA⁺ strains are more prone to gastritis than those infected with strains that do not express this protein^[41]. VacA may interfere with phagocytosis and antigen presentation^[42,43], reducing the activation of Jurkat cells, thereby inhibiting the activation of NFAT, an important transcription factor that is necessary for the expression of genes involved in the expansion of T cells activated by bacterial antigens^[44], thereby ensuring the evasion of *H. pylori* from the adaptive immune response.

The BabA and SabA adhesins are encoded by the *babA* and *sabA* genes that encodes an outer membrane protein, BabA, which binds to the type B blood group antigen in gastric cells^[1], while sabA binds to the sialyl-Lewis x/a antigens^[45]. The adhesion of bacteria to the gastric epithelium allowing the release of the CagA and VacA factors into the host cells is mediated by BabA, which facilitates colonization, induces mucosal inflammation and can influence the severity of the disease^[46,47]. *H. pylori* strains which carry *babA*, *vacAs1* and *cagA* together are associated with duodenal ulcer and present a higher risk of gastric cancer^[48]. The inflammatory response may be increased due to sabA-mediated adhesion, by facilitating the utilization of nutrients exudated from damaged host

cells. Thus, as the inflammatory response increases, the *sabA* expression may be switched off, allowing the contact between the bacteria and the inflamed epithelium to be broken, thus maintaining prolonged infection^[45]. However, there is no clinical or epidemiological evidence associating *sabA* to gastric cancer. Another gene that encodes an outer membrane protein is *oipA*, located near *cagPAI*^[49]. It is regulated by a slipped-strand repair mechanism based on the number of Cysteine-Threonine dinucleotide repeats in the 5' regions of the gene^[49]. The *oipA* gene has the ability to induce interleukin (IL)-8 from gastric epithelial cells, as *cagA* and its status have been linked to the discrimination of duodenal ulcer and gastritis^[49,50].

The *dupA* gene, located in the plasticity region of the *H. pylori* genome, represents a marker of virulence with pathogenic potential^[51]. This gene was reported to be associated with increased risk of duodenal ulcer^[51], with lower gastric cancer incidence and lower acid output, including patients with peptic ulcer^[52]. As opposed to these findings, there are studies of *dupA* status in which no association with any gastroduodenal disease was found^[53].

The *iceA* gene, another virulence factor, has two variants, *iceA1* and *iceA2*^[54]. However, the function of *iceA2* remains undefined^[55], while the expression of *iceA1* is increased in some populations by the contact of *H. pylori* with human gastric epithelial cells and is associated with peptic ulcer^[56]. Nevertheless, the development of erosive gastritis has been related to strains carrying genes *iceA1*, *cagA* and *vacAs1a/m1*, while enanthematous gastritis is associated with *vacAs2/m2* and *iceA2* genotypes^[57]. Moreover, the severity of gastritis is related with the coexistence of the *iceA2* gene with *cagA*, *vacAs1/m1* and *babA2*^[58].

In this review, we first approached about the *H. pylori* infection and its virulence factors, topics widely addressed in other recent reviews^[2,18,19]. Thus, we will focus mainly on modulation of host immune response triggered by *H. pylori*, and the advances in the fast developing field of gene expression profiles in gastric mucosa, which can change as a consequence of *H. pylori* infection, leading to the activation of transcription of genes involved in defense mechanisms, inflammatory and immunological responses, cell proliferation and apoptosis. Moreover, we highlighted the importance of the eradication of *H. pylori*, which plays an important role in the restoration of gastric mucosa inflammation and on gene expression levels. In light of the increasing involvement of microRNAs (miRNAs) in the regulation of posttranscriptional gene silencing, we addressed the action of different miRNAs in precancerous lesions, gastric cancer, and inflammatory processes induced by *H. pylori*, evidencing its participation in several steps of gastric carcinogenesis.

MODULATION OF *H. PYLORI*-TRIGGERED HOST IMMUNE RESPONSE

As soon as *H. pylori* bacteria colonize the stomach, the epithelial cells and their innate immune receptors, mainly the toll-like receptors (TLRs)^[19], recognize the bacteria (Figure 1). This attachment process can be facilitated

by the action of adhesins (SabA and BabA) expressed by bacteria, which favor the action of other virulence factors (CagA and VacA). Soon after, the host's innate and adaptive immune systems are activated, leading to the recruitment of a wide variety of inflammatory cells and mediators, and the activation of transcription factor nuclear factor (NF)- κ B and pro- and anti-inflammatory cytokines, cell proliferation and survival factors. The activation of the immune system in response to the presence of the bacteria increases the production of reactive oxygen and nitrogen species [reactive nitrogen species (RNS) and reactive oxygen species (ROS)] by increasing oxidative/genotoxic stress, which can cause cell and DNA damage, favoring the appearance of mutations that may facilitate the carcinogenic process. In addition, the expression of the immune response mediators can be regulated by miRNAs, and inflammatory mediators can change the miRNAs expression^[59-61].

Members of the TLR family are essential components of the innate and adaptive immune response and comprise 10 types in humans, TLR1 to TLR10^[62]. They recognize molecular structures of pathogenic microbe-associated molecular patterns (PAMPs), like lipopolysaccharides (LPS), lipoproteins, lipoteichoic acid, peptidoglycan, lipoarabinomannan and flagellin^[63]. *H. pylori* LPS, as cell wall components, are recognized mainly by TLR4; however modifications of the LPS structure can alter this recognition and poorly stimulate the host immune response, enhancing the bacterial evasion and pathogenicity^[64]. *H. pylori* is also recognized by TLR2 through other forms of LPS structurally different from those recognized by TLR4^[65]. TLRs are dependent on the presence of MyD88 (myeloid differentiation primary-response gene 88) for efficient signal transduction. The MyD88 complex is associated with interleukin-1-receptor-associated kinase-1 (IRAK1) and IRAK4. IRAK1 is phosphorylated and then dissociated from MyD88. Subsequent dissociation of protein complexes occur by phosphorylation, and, as the last step, NF- κ B is translocated into the nucleus, activating the expression of genes related to the inflammatory process^[66] (Figure 1A).

CagA-positive strains contribute to the inflammatory response, since this virulence factor causes an increase in the production of certain cytokines such as IL-1 β and IL-8^[67,68] and activation of NF- κ B, which can confer a proliferative phenotype to the bacteria, important in the process of carcinogenesis^[69], promoting induction of growth factors and suppression of apoptosis^[70]. Thus, CagA deregulates the cell signaling pathways and favors the arising of oncogenic cells, which is important in the pathogenesis of *H. pylori*^[71]. The VacA factor induces a pro-inflammatory response^[72] and multiple cellular activities that facilitate chronic colonization of the gastric mucosa by bacteria^[68]. A recent study showed that overexpression of VacA led to the production of tumor necrosis factor (TNF)- α , IL-1 β , nitric oxide, reactive oxygen species and the activation of NF- κ B, which can be associated to pro-inflammatory cytokines and cell apoptosis^[73]. VacA also can affect the immune system

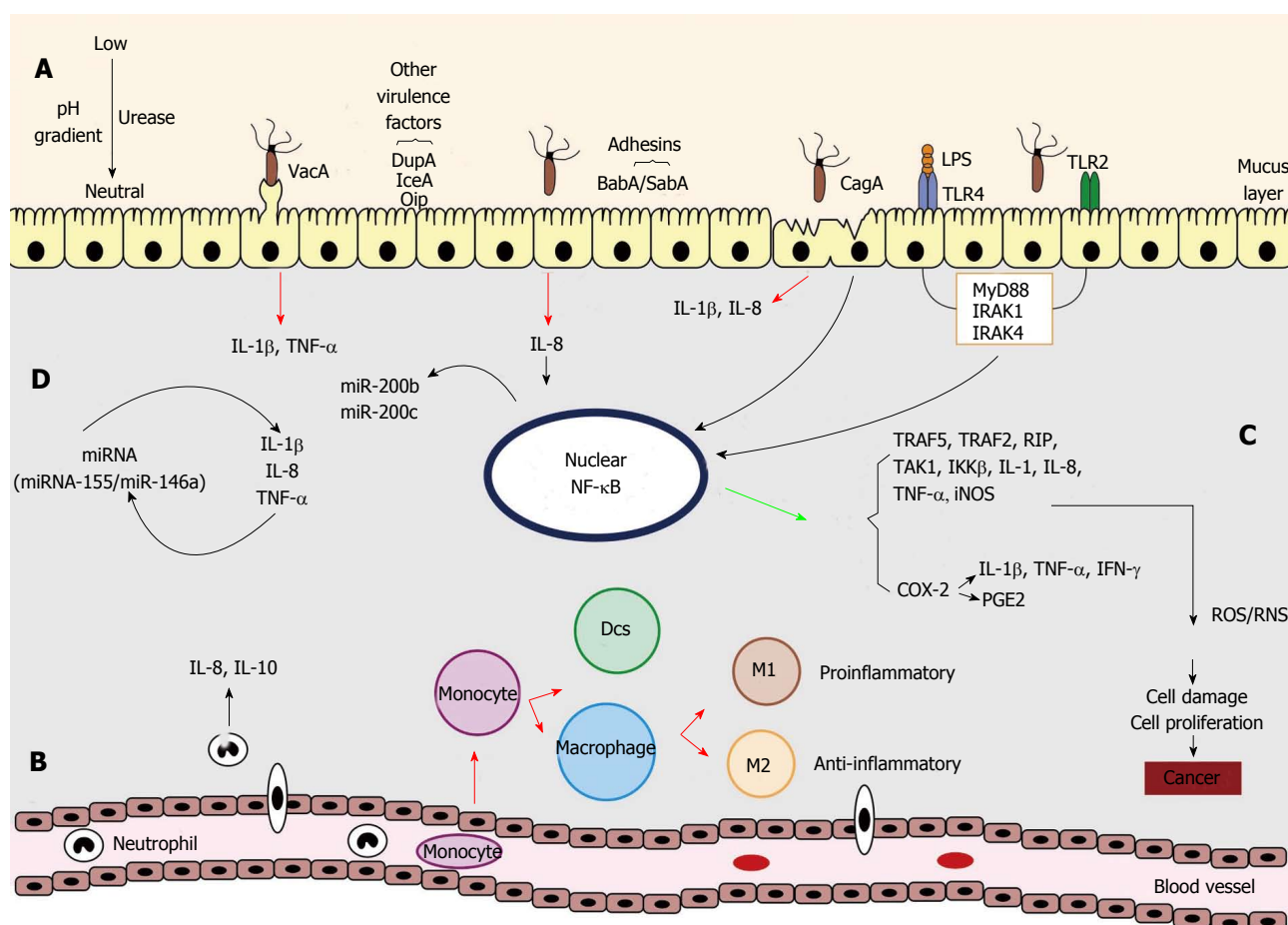


Figure 1 Pathogenesis of *Helicobacter pylori* infection and host immune response. A: Bacterial urease neutralizes the gastric pH, enabling the colonization of gastric epithelial cells by the bacteria and their motility in the mucus layer. Adhesion of the bacteria to the gastric epithelium is mediated by BabA and SabA adhesins, allowing the release of factors CagA and VacA into the host cells, which causes a strong systemic immune response and inflammation of the gastric mucosa. *Helicobacter pylori* LPS is recognized by toll-like receptors, mainly TLR4 and TLR2, in cooperation with the adapter molecule MyD88 associated with IRAK1 and IRAK4 that leads to activation of transcription factor NF- κ B, activating inflammatory signaling pathways; B: The immune response is also activated, with the recruitment of inflammatory cells at the infection site, inducing the production of various pro- and anti-inflammatory mediators; C: After NF- κ B activation, rapid expression of multiple pro-inflammatory cytokines, chemokines such as the tumor necrosis factor alpha (TNF- α) and interleukins, and consequently activation of oncogenic pathways may culminate in cancer; D: The expression of some miRNAs is changed by *H. pylori* infection and the host immune response is regulated accordingly. LPS: Lipopolysaccharides; IL: Interleukin; COX-2: Cyclooxygenase; RNS: Reactive nitrogen species; ROS: Reactive oxygen species; IFN: Interferon.

enabling *H. pylori* evade the adaptive immune response to establish persistent infection, since can interfere with phagocytosis and antigen presentation and also inhibit T cell proliferation^[24].

H. pylori infection, after activation of NF- κ B and cytokines production, causes chemotaxis of monocytes/macrophages and infiltration of polymorphonuclear leukocytes^[74], recruitment of neutrophils and lymphocytes^[75] that also induces the production of IL-8 and IL-10 by neutrophils^[76] (Figure 1B). In this pathway, monocytes secrete interleukins such as IL-1 β , IL-6, IL-10, and IL-12p40 (partially secreted as IL-23), dendritic cells (DCs) secrete IL-1 β , IL-6, IL-10, IL-12p40, IL-12p70, and IL-23, while M1 macrophages produce mainly IL-1 β , IL-6, IL-10, IL-12p40 and IL-23. M2 macrophages synthesize IL-10 but produce less pro-inflammatory cytokines than M1 macrophages, which can control inflammation, leading to a chronic inflammatory response^[77].

The activation of DCs and M1 macrophages is correlated with an increased capacity to induce T-cell prolifera-

tion like T helper cells and decreased phagocytosis^[78,79], as well as Th17 that can promote chronic infection triggered by a chemotaxis system^[80]. Even though *H. pylori* avoids phagocytosis and prevents the induction of an adaptive immune response, macrophages engulf the bacterium but cannot kill it, which facilitates the chronic infection^[81]. This infection results in a predominantly T cell-mediated immunity rather than humoral immunity, with Th1 and Th17 responses, which increase the production of IL-1 β , TNF- α and IL-8^[82,83]. While Th17 cell differentiation is promoted by TNF- α and IL-6 from activated macrophages/dendritic cells, Th1 cell development is triggered by IL-12 and interferon (IFN)- γ ^[2]. In addition, the recruitment of antigen-specific regulatory T cells has also been reported, facilitating the permanent colonization of the stomach through direct cell-to-cell contact or by secreting cytokines [transforming growth factor (TGF)- β 1 and IL-10] that modulate the immune response^[84].

Moreover, NF- κ B regulates the expression of several genes, for example *TRAF5*, *TRAF2*, *RIP*, *TAK1*

and IKK β , some of which are associated with inflammation and cancer^[85]. Its activation by LPS leads to the synthesis of IL-1, IL-8, IL-10 and TNF- α , and iNOS (Figure 1C). NF- κ B also upregulates the expression of the pro-inflammatory cyclooxygenase (COX-2) enzyme, whose function is to induce cytokines such as TNF- α , interferon- γ and IL-1, inhibiting apoptosis, maintaining cell proliferation and stimulating angiogenesis in favor of carcinogenesis^[20]. In *H. pylori*-associated gastric cancer there are reports showing increased expression of COX-2 and prostaglandin E2 activated by TLR2/TLR9 and NF- κ B, and this induction is mediated by the activation of the epidermal growth factor receptor in gastric epithelial cells^[86,87]. Added to inflammatory stress effects, influences on the cell cycle and cell polarity, *H. pylori* also activates multiple oncogenic mechanisms, such as the PI3K/AKT/GSK3 β pathway that regulates many functions like cell growth, proliferation, differentiation and motility, and its aberrant activation is associated with various types of cancer, including stomach cancer^[88,89]. The presence of these bacteria also affects the STAT3 protein pathway that regulates cell growth, differentiation and apoptosis, in which a high expression of STAT3 is associated with advanced stage and poor prognosis of gastric cancer^[90]. All the high immune stimulation produced by these molecules results in the production of ROS and RNS by neutrophils attracted to the infection site, which can cause cell damage, leading to gene mutations and cell proliferation, favoring the emergence of gastric cancer^[91].

Other important members of the class of immune regulators are miRNA^[92]. Recent reports have highlighted the regulatory role of miRNAs in *H. pylori* infection and associated diseases (Figure 1D). For example, a strong inflammatory response characterized by the early production of pro-inflammatory TNF- α and IL-6 cytokines, followed by IL-10, IL-1 β and IL-23 secretion as a consequence of miR-146a up-regulation and strong miR-155 induction, which raised the TNF- α production^[93]. In contrast, IL-8, TNF- α and IL-1 β could contribute to the induction of miR-146a during *H. pylori* infection^[94]. Therefore, miRNAs modulate the *H. pylori* infection and are also affected by these bacteria, as, for example, the synthesis of the transcription factor NF- κ B that can act as a transactivator of miR-200b and miR-200c^[60]. This issue will be discussed in more detail in the last section of this review. Thus, all the pathways reported above show the need for new approaches in order to reach a better understanding of the influence of *H. pylori* on the host immune system, allowing the working out of preventive measures and efficient new strategies of *H. pylori* eradication.

H. PYLORI INFECTION DEREGULATES THE EXPRESSION OF GENES INVOLVED IN INFLAMMATORY RESPONSE AND CELL KINETICS

In addition to a marked inflammatory response of

the host, activation of signaling pathways and gastric mucosa injury, *H. pylori* infection can enhance cell proliferation and apoptosis of gastric epithelial cells^[95]. Thus, to counteract *H. pylori* infection, the host activates gene transcription involved in his defense mechanisms, inflammatory and immunological response and control of cell kinetics^[31,96,88]. Gene expression profiling analysis in gastric biopsies and cell lines in response to *H. pylori* infection might be one approach to better understand the role of important factors involved in the pathogenic mechanism of these bacteria.

In this respect, Hofman *et al.*^[97] (2007) evaluated the gene expression profile of the gastric mucosa of *H. pylori*-infected compared to noninfected patients and highlighted a distinct transcriptional pattern in biopsies of the antral and fundic regions, associated also with bacterial density and virulence factors such as *cagA*, *vacA* and *babA2*. The authors reported up-regulation in receptors and co-receptors involved in bacterial recognition such as *TLR2*, *TLR4*, *LY96*, *ITGB2*, *VCAM1*, *MAPK8*, *RAC2*, *SLA*, *ADAM*, *MMP*, *IFITM1* and *PAP*, signal transduction, inflammation and immune response, proteolysis, apoptosis and cell proliferation in antral biopsies from infected patients in comparison with biopsies from noninfected individuals. It was also observed that several transcripts encoding chemokines and their receptors were up-regulated in response to *H. pylori* infection. More recently, microarray data of gene expression profiling in gastric antral mucosa from chronic superficial gastritis patients infected by *H. pylori* and uninfected subjects revealed 38 differentially expressed genes, including 23 up-regulated and 15 down-regulated genes related to protein metabolism, inflammatory and immunological reaction, signal transduction, gene transcription and trace element metabolism^[98]. These data indicate that *H. pylori* infection could induce carcinogenesis by altering cellular gene expression processes, evade the host defense mechanism, increase inflammatory and immune responses, activate NF- κ B and Wnt/ β -catenin signaling pathways, and disturb the metal ion homeostasis. However, the functional significance of these selected genes needs to be further evaluated in other studies.

TLRs expression has been evaluated in *H. pylori* infection due to its relevant role in the recognition of pathogenic components such as bacterial LPS. In *H. pylori*-negative normal gastric mucosa, *TLR5* mRNA is the most expressed, followed by *TLR2* and *TLR4*, whereas in *H. pylori*-infected normal gastric mucosa, intestinal metaplasia, independently of *H. pylori* infection, and in the dysplasia/cancer sequence *TLR2* and *TLR4* are the most overexpressed^[99]. Therefore, these findings suggest that progressive activation of these receptors, initially by *H. pylori*, but also by other PAMPs or damage-associated molecular patterns at later stages, may have an important role in gastric carcinogenesis and tumor progression^[99]. However there is also indication of no quantitative differences in the *TLR4* and *TLR5* mRNA levels, regardless of the presence or absence of *H. pylori*, in both gastric epithelial cell biopsies and AGS cells, suggesting that the

mRNA levels of these receptors may not be influenced by the infection process, or at least not at the time points selected for analysis^[100].

H. pylori-CagA⁺ strains often trigger more potent inflammatory and immune responses, leading to a more severe disease, which may be mediated by nucleotide oligomerization domain 1 (NOD1) by recognizing the intracellular pathogen and initiating pro-inflammatory signaling cascades^[101,102]. Gastric epithelial cells co-cultured with *H. pylori*-CagA⁺ strains show increased production of IFN- γ -inducible chemokines, IP-10 and MIG, in response to IFN- γ stimulation. In addition, gastric biopsies from infected and non-infected patients with gastritis or gastric cancer show increased mRNA expression levels of *NOD1*, *CXCL8*, *IRF1* and *CXCL10*, when compared with normal tissue^[103]. Likewise, up-regulation of pro-inflammatory molecules expression also occurs in gastric tumor tissues compared to matched non-tumor samples such as *IRF1*, *NOD1* and *CXCL8*. Thus it is proposed that NOD1 and the IFN- γ signaling pathway regulate the expression levels of the tumor suppressor gene *IRF1*. That could, in some instances, potentiate oncogenic changes in the gastric mucosa as a consequence of infection with virulent *H. pylori*-CagA⁺ strains and exacerbate disease severity and progression during chronic *H. pylori* infection.

In addition, *H. pylori*-CagA⁺ strains also appear to be related with differential activation of two signaling proteins, STAT3 and ERK1/2 in gastritis patients^[104]. The differential activation of these two signaling proteins may in part explain the increased predisposition to gastric cancer when infected with *H. pylori*-CagA⁺ strains compared to their CagA⁻ counterparts, due to the activation of epithelial cell turnover, thus increasing the likelihood of gaining somatic mutations and subsequent cellular transformation. Recently, in AGS cells incubated with *H. pylori*-CagA⁺ strains 147A⁻ and 147C was observed specific and significant alterations in gene expression profiles^[105]. Up-regulated genes primarily encoded signal transduction (23.2%), transport (13.8%), transcription (12.6%), metabolic (11.3%), immune and inflammatory responses (6.9%), adhesion and migration (5.9%), and development proteins (5.0%), while down-regulated genes encoded metabolic (16.1%), transcription (14.6%), transport (14.6%), signal transduction (10.6%), translation (5.9%), cell cycle (5.1%), and apoptosis (3.0%). Among the differentially expressed genes compared to non-treated AGS cells, the *EMT* (epithelial-mesenchymal transition) gene was selected because it seems to facilitate the invasion of cancerous cells into both local and distant tissues. Thus, the *H. pylori*-CagA⁺ strain plays a significant role in epithelial-mesenchymal transition, so the prevention of *H. pylori*-CagA⁺ infection may be an effective approach in preventing the progression or metastasis of tumor cells that occurs *via* EMT-inducing genes.

Considering the role of *H. pylori* infection as a key event in triggering all these changes in gene expression of the infected gastric mucosa, and even the risk of ma-

lignant progression, the eradication of these bacteria has been recommended in various countries^[106]. Once the gastric colonization by the pathogen is rarely eliminated spontaneously, *H. pylori* eradication is regarded as a first-line therapy to reverse the pre-neoplastic lesions and prevent malignant progression^[107]. The standard triple treatment regimen of infection consists of two or three antibiotics (amoxicillin or clarithromycin) and a proton pump inhibitor, associated or not with bismuth salts, for 1 or 2 wk^[108], reaching an eradication rate higher than 90%^[109,110].

Although the eradication of *H. pylori* can result in partial regression of pre-neoplastic lesions, to this date few studies have evaluated the role of treatment for the restoration of gastric mucosa inflammation and normalization of gene expression levels. Tsai *et al.*^[107] (2006) employed microarray technology to investigate changes in gene expression profiles using samples from a double-blinded, placebo-controlled clinical trial, associated with *H. pylori* infection and eradication of the bacteria. One year after the bacteria eradication therapy, were identified 30 genes whose expression was significantly down-regulated, the majority of which were associated with immune response and inflammation (*CXCL1*, *CXCL14*, *IGLC2*, *LOC400986*, *TNFSF10* and *OAS1*), while in the placebo group the expression of 55 genes differed significantly in the same period (32 up-regulated and 23 down-regulated). Among them, genes involved in cell-cell adhesion and lining, cell cycle differentiation, and lipid metabolism and transport were down-regulated over time in the treatment group but up-regulated in the placebo group. Taken together, these findings showed that *H. pylori* infection and its subsequent eradication resulted in alterations of gene expression associated with cell damage, inflammation, proliferation, apoptosis and intestinal differentiation, suggesting that *H. pylori* eradication may stop or reverse ongoing malignancy-related molecular processes in the stomach. In this respect, further studies are needed to evaluate the use of these genes as possible markers for gastric cancer risk.

The eradication therapy also appears to influence the expression of the transcription factor FOXP3 by CD4⁺CD25 regulatory T cells in the gastric and duodenal mucosa leading to reduced expression in response to treatment^[111]. Moreover, was observed a decrease of IFN- γ and *IL-10* gene expression in the antral mucosa after eradication of *H. pylori*. Thus, it is possible that in the infected mucosa the overall immune response may be shifted towards an anti-inflammatory response. This could indicate that a moderate regulatory mechanism is induced in the presence of the bacteria, keeping an immunologic balance where the inflammation is maintained at a controlled level by the suppressive regulatory T cells. This effect may explain why *H. pylori* infections become chronic.

The effect of *H. pylori* eradication therapy was also observed on receptors expression levels such as genes human beta defensin 2 (*hBD2*) and *hBD3*, which codify antimicrobial peptides on the mucosal surface and act in

Table 1 MicroRNAs involved in *Helicobacter pylori*-induced inflammatory and carcinogenic processes

miRNAs	Regulation	Targets and action	Ref.
let-7b	Down	<i>TLR4, NF-κB, COX-2, Cyclin D1, IL1B</i> Initiation of immune response	[128,129]
has-miR-17	ND	ND	[130]
hsa-miR-21	Up	<i>RECK, TGFBR1, TGFBR2</i> Promotes proliferation, migration and invasion, inhibits apoptosis	[123,130]
hsa-miR-25	Up	ND	[130]
has-miR-93	Up	ND	[130]
has-miR-103	Down	TNFα ND	[128]
hsa-miR-146a	Up	<i>IRAK1, CARD10, COPS8, PTGS2</i> Inhibits tumor-promotes cytokines and growth factors	[131,132]
hsa-miR-155	Up	<i>SMAD2</i> Attenuation of the inflammatory response	[133]
hsa-miR-194	Up	ND ND	[130]
hsa-miR-196	Up	ND	[130]
hsa-miR-200b	Up	<i>ZEB1</i> Promotes EMT	[60]
has-miR-200c	Up	<i>ZEB1, IL6</i> ND	[60,128]
has-miR-222	Up	<i>RECK</i> Promotes cell proliferation	[134]
has-miR-223	Up	<i>IL6, IL1B,</i> ND	[135,136]
hsa-miR-370	Down	<i>FoxM1</i> Promotes proliferation	[137]
has-miR-371-5p	Down	<i>LATS2</i> Inhibits cell cycle progression	[138]
has-miR-372	Down	<i>LATS2</i> Inhibits cell cycle progression	[138]
has-miR-373	Down	<i>LATS2</i> Inhibits cell cycle progression	[138]
hsa-miR-375	Down	<i>IL8</i> ND	[128]
has-miR-449b	Down	<i>MET, GMNN, CCNE2, SIRT1, HDAC1</i> Promotes proliferation Inhibits senescence and apoptosis	[139]
has-miR-584	Up	<i>PPP2a, Foxa1</i> Promotes EMT and stem cells differentiation	[140]
hsa-miR-1290	Up	<i>NKRF, Foxa1</i> Promotes EMT and stem cells differentiation	[140]

EMT: Epithelial to mesenchymal transitions; ND: Not determined (in gastric mucosa); NF: Nuclear factor; COX-2: Cyclooxygenase; TNF: Tumor necrosis factor.

the innate immune responses to human pathogens^[112]. Up-regulation of both *hBD2* and *hBD3* transcripts were observed in *H. pylori*-positive subjects that correlated with the degree of gastritis in corpus and antrum. However, after successful eradication therapy, while the mucosal *hBD2* transcript levels returned to normal, the *hBD3* protein expression level remained unchanged. In addition, while infiltrating granulocytes disappeared completely,

higher lymphocytic infiltration still persisted compared to *H. pylori*-negative subjects^[112]. Possibly *H. pylori*-positive patients were most likely infected in their early childhood and had carried the bacteria for decades, speculating whether the decreased expression of *hBD3* after 3 mo of treatment should be attributed to long-lasting effects on the epithelial cells that had not been completely renewed or to the lymphocytic infiltration still present at the time of study.

In a broader perspective, despite the still limited studies on the role of *H. pylori* eradication in the normalization of gene expression levels in gastric mucosa, such studies showing genes with significant changes of expression over time may help reveal molecular markers involved in inflammatory processes and mechanisms of progression from precancerous lesions to malignancy.

MIRNAS REGULATING THE INFLAMMATORY AND CARCINOGENIC PROCESSES INDUCED BY *H. PYLORI*

miRNAs, non-coding ribonucleic acids with about 22 nucleotides^[113], are involved in the process of post-transcriptional gene silencing through the pairing with mRNA target, promoting its degradation^[114,115] or, mostly in animals, causing repression of mRNA translation^[116,117]. Since the discovery of miRNAs, their key role in the regulation of gene expression^[118,119] and their participation in various cellular and systemic functions, they have been associated with various pathologies, such as inflammation and cancer^[120,121].

The expression of miRNAs is tissue-specific and they have different cellular functions, such as regulation of proliferation, apoptosis^[122,123], differentiation^[124,125] and carcinogenesis, and can be used as biomarkers for tumor origin^[120,126]. With particular regard to the stomach, there are various studies reporting different miRNAs in normal mucosa^[127], *H. pylori*-induced precancerous lesions and gastric cancer (Table 1)^[60,123,128-140]. Studies on miRNA in precancerous gastric lesions are still scarce. For example, chronic gastritis experimentally induced by *H. pylori* showed the action of hsa-miR-155 in regulating the response of Th1 and Th17 cells to control infection and, in the meantime, induced precancerous pathologies associated with this bacterium by IFN-γ production^[121,141]. In intestinal metaplasia was demonstrated that the CagA bacterial protein stimulates the expression of hsa-miR-584 and hsa-miR-1290, which results in down-regulation of the forkhead box A1 (*Foxa1*) gene, thus inducing transdifferentiation of gastric epithelial cells^[140].

In *H. pylori*-associated gastric cancer, an increasing number of studies have described the occurrence of deregulation of miRNA expression and its involvement in the regulation of gene expression. *H. pylori* and CagA genotype inhibit has-miR-370 expression in both gastritis and gastric cancer, which led to overexpression of this target *FoxM1*. This increased expression was gradual from inflammation to cancer, resulting in cell proliferation for

gastric carcinogenesis^[137]. In gastric cancer cell line, non-malignant gastric cell line, as well as in human gastric mucosal tissue, *H. pylori* is able to increase expression of has-miR-222 promoting cell proliferation by gradually decrease the expression of their target *RECK*^[134], so *H. pylori* infection can induce carcinogenesis through altering expression of some miRNAs. Also *H. pylori*-infected AGS cell line results in the repression of hsa-miR-371-5p, hsa-miR-372 and hsa-miR-373, which leads to the inhibition of cell cycle progression by up-regulation of their target *LATS2* (serine-threonine kinase)^[138]. hsa-miR-200b and hsa-miR-200c that have a common target, *ZEB1*, are transactivated by transcription factor NF- κ B due to the presence of the *cagA* genotype, so that the gastric epithelial cells begin to undergo mesenchymal transition^[60].

Considering the importance of the treatment and eradication of *H. pylori* to restore gastric tissue homeostasis, Matsushima *et al*^[135] (2011) found 31 miRNAs differentially expressed in infected- noncancerous gastric mucosa compared to non-infected individuals. Of these miRNAs, only has-miR-223 showed increased expression in *H. pylori*-positive individuals. In a subgroup of four patients in which *H. pylori* was eradicated, was observed that 14 miRNAs that were down-regulated in the presence of the pathogen had their levels increased after four weeks of eradication therapy. However, in a patient in whom the therapy was not satisfactory, the levels of these miRNAs were unaltered^[135]. However, eradication of the bacteria year after treatment did not change the expression of oncogenic miRNAs in metaplastic glands, but it was decreased in non-metaplastic glands, indicating that the treatment was effective in restoring the miRNAs expression only in the early stages of gastric transformation^[130]. In addition, hsa-miR-21, hsa-miR-25, hsa-miR-93, hsa-miR-194 and hsa-miR-196 were overexpressed in gastric cancer in comparison to *H. pylori*-positive gastric ulcer or atrophic gastritis, and the eradication decreased the expression of these miRNAs only in atrophic gastritis^[130]. These findings evidence that *H. pylori* is able to change the expression of miRNAs in noncancerous gastric mucosa, and this is one of the possible mechanisms for manipulating the host response.

H. pylori can remain in the stomach at high density levels and for a long time, indicating that the host immune response is not effective in eliminating the pathogen. This may be due to the deregulation caused by the bacteria in the expression pattern of miRNAs which target cytokines and other mediators of the immune response. The miRNA has-miR-21 is a possible regulator of *H. pylori*-induced inflammation, targeting the receptor of the TGF β signaling pathway (TGF β R1 and TGF β R2)^[142], and the mature form of this miRNA shows increased expression in both gastric cancer and *H. pylori*-infected gastric tissue^[123]. hsa-miR-155 and hsa-miR-146a are also involved in the attenuation of the inflammatory response against *H. pylori*. In this process, the MyD88 complex and adaptor proteins (IRAK-1 and TRAF6) of the TLRs signaling cascade are targeted by these miRNAs, resulting in decreased NF- κ B activation. In contrast, *H. pylori* also

up-regulates hsa-miR-155 expression, which occurs in an NF- κ B-dependent manner, resulting in decreased levels of pro-inflammatory mediators IL-8 and growth-related oncogene- α ^[131-133]. Moreover, *H. pylori* infection decreases the expression of let-7b, increasing the production of TLR4, NF- κ B, COX-2 and Cyclin D1, thus contributing to the initiation of the immune response and the inflammation of the gastric mucosa^[129]. Particularly, Isomoto *et al*^[128] (2012) investigated the association of various miRNAs with cytokine expression in *H. pylori*-positive gastric mucosa and found a negative correlation among let-7b, hsa-miR-200c, hsa-miR-375 and hsa-miR-103 and interleukins IL-1 β , IL-6, IL-8 and TNF- α , respectively. Other relationships between inflammatory mediators and miRNAs are described, as for example has-miR-370 and reduced expression of TGF β R2^[143], has-miR-365 and negative regulation of IL-6^[144], and has-miR-223 and the reduction of IL-6 and IL-1 β ^[136].

Therefore, inflammatory process induced by *H. pylori* leading to precancerous gastric lesions and gastric cancer can alter the expression pattern of miRNAs in order to influence biological processes by changing the expression of mRNA targets. Eradication of the bacteria may be a strategy for restoring normal levels of these miRNAs in the gastric mucosa at early stages of malignant transformation, reducing the risk of gastric cancer.

CONCLUSION

After millennia of co-evolution of *H. pylori* bacteria with human hosts, complex mechanisms of interaction between pathogen and host developed, allowing its persistence and subversion of the immune system and successful colonization in the human stomach. Numerous studies about colonization and adhesion of bacteria in gastric epithelial cells, diversity of virulence factors, activation of signaling pathways, evasion and subversion of the immune system and, more recently, about changes in the gene expression profile of infected mucosa and participation of miRNAs have contributed to a better understanding of the host-pathogen relation. Taken together, these data may help to clarify pivotal biological and molecular mechanisms of infection pathogenesis and to identify clinically significant biomarkers, with the possibility of disclosing novel therapeutic targets for treatment strategies, especially in patients who developed resistance mechanisms. Taking into account that *H. pylori* infection is a relevant risk factor for the development of gastric cancer, strategies aiming for a better understanding of the mechanisms involved in its pathogenesis and effective eradication therapies are critical for the prevention of this type of malignancy.

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