

REVIEW ARTICLE

Persistent colonization of *Helicobacter pylori* in human gut induces gastroduodenal diseases

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ABSTRACT

Helicobacter pylori are gut bacteria colonize in the epithelial cell lining of the stomach and persist there for long duration. Around two-thirds of the world's populations are infected with *H. pylori* and cause more than 90 percent of ulcers. The development of persistent inflammation is the main cause of chronic gastritis that finally results in a severe consequence known as stomach cancer. Two major virulence factors cytotoxin-associated gene product (cagA) and the vacuolating toxin (vacA) are mostly investigated as their close association with gastric carcinoma. In this review, host immunity against *H. pylori* infection and their evasion mechanism are intensely explored. It is the fact, that understanding pin point molecular mechanisms of any infection is critical to develop novel strategies to prevent pertinent diseases. *J Microbiol Infect Dis* 2014; 4(4): 170-176

Key words: vacA, cagA, miRNA, COX-2, Gastric carcinoma

İnsan barsağında gastrodüedonal hastalıklara yol açan *Helicobacter pylori*'nin kalıcı kolonizasyonu

ÖZET

Helicobacter pylori midenin epitelyal hücre hattında kolonize olan bir barsak bakterisi olup burada uzun süre sebat eder. Dünya nüfusunun yaklaşık üçte ikisi *H. pylori* ile enfektedir ve ülserlerin % 90'ından fazlasının etkenidir. Devamlı enflamasyonun gelişmesi kronik gastritin başlıca sebebidir ve nihai olarak mide kanseri olarak bilinen ciddi sonuçlara yol açabilir. Mide karsinomu ile yakından ilişkili olan sitokine eşlik eden gen ürünü (cagA) ve vakuoleştirici toksin (vacA) en fazla araştırılan iki major virulans faktörüdür. Bu derlemede *H. pylori*'ye karşı konak immünitesi ve onların kurtulma mekanizmaları yoğun şekilde açıklandı. Gerçektende bir enfeksiyonun bağlanmasındaki moleküler mekanizmasını anlamak korunma için yeni stratejiler geliştirmede kritik öneme sahiptir.

Anahtar Kelimeler: vacA, cagA, miRNA, COX-2, Mide karsinomu

INTRODUCTION

Helicobacter pylori, (*H. pylori*), is a spiral-shaped bacterium residing in the mucus layer of human stomach. *H. pylori* secrete the enzyme urease to survive in the highly acidic condition of the stomach, as it converts urea to ammonia. Ammonia reduces the acidity, making bacteria friendly stomach environment. In addition, the helical shape of *H. pylori* allows it to dig into the mucus layer, which is less acidic than the inner area, or lumen, of the stomach. *H. pylori* can also stick to the cells that line up the

inner surface of the stomach. Immune cells accumulate near sites of *H. pylori* infection in order to kill invading bacteria. They cannot prevent stomach lining erosion caused by the bacterium. Additionally, *H. pylori* interfere with local immune responses, making them unsuccessful in eliminating the bacteria.^{1,2}

Host-pathogen interaction

The entire *H. pylori* genome consists of ~1.65 million nucleotide and encodes about one and half

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thousand proteins.³ The discovery of a large family of 32 associated outer-membrane proteins (Hop proteins) and the discovery of many genes that can be regulated by slipped strand mispairing-mediated mutagenesis were the most significant findings of two *H. pylori* genome-sequencing projects.⁴ Such phase-variable genes encode enzymes that alter the antigenic structure of surface molecules, support the entrance of foreign DNA into the bacteria, and accelerate bacterial motility.⁵ During persistent or transient mixed infections, *H. pylori* modifies its genome continuously by importing small pieces of foreign DNA from other *H. pylori*.⁶

Most of the *H. pylori* strains use an auto transporter mechanism to express the vacuolating cytotoxin VacA (95-kD), a secreted exotoxin.^{7,8} The toxin undergoes self-insertion into epithelial-cell membrane forming a hexameric anion-selective and voltage dependent channel through which bicarbonate and organic anions can be transported, providing nutrients to the bacterium.^{9,10} VacA also causes release of cytochrome c from mitochondrial membrane inducing apoptosis.¹¹

Most of the VacA localizes on epithelial cell membrane and oligomerizes constructing an anion specific channels across the membrane. Overall impact of functional VacA on epithelial cells comprises modification of delayed endocytic cubicle, magnifying membrane permeability, and finally augmentation of apoptosis.¹² Since S2 was found well conserved with VacA as it must have similar function which is still undefined.^{13,14}

Mostly, *H. pylori* strains have the *cag* pathogenicity island (*cag* PAI), a 37-kb genomic portion containing 29 genes (Figure 1). The *cagA* (cytotoxin-associated gene A) gene is the most widely studied of all *H. pylori* genes.^{15,16} The 3' region of the *cagA* gene is variable that is responsible for increased risk for gastric cancer and greater susceptibility to pH. Several of these express constituents of a predicted type IV secretion apparatus that translocates the CagA protein (120-kD) into the host cell.¹⁷ The *cagA* gene flanks 40kb genomic region having 41 putative genes of the *cag* pathogenicity island (PAI) in *H. pylori*. These genes have remarkable role in increase of inflammation and secretion of virulence-related products, like IL-8; recruitment of neutrophil, phosphorylation of tyrosine, and release of protein.¹⁸

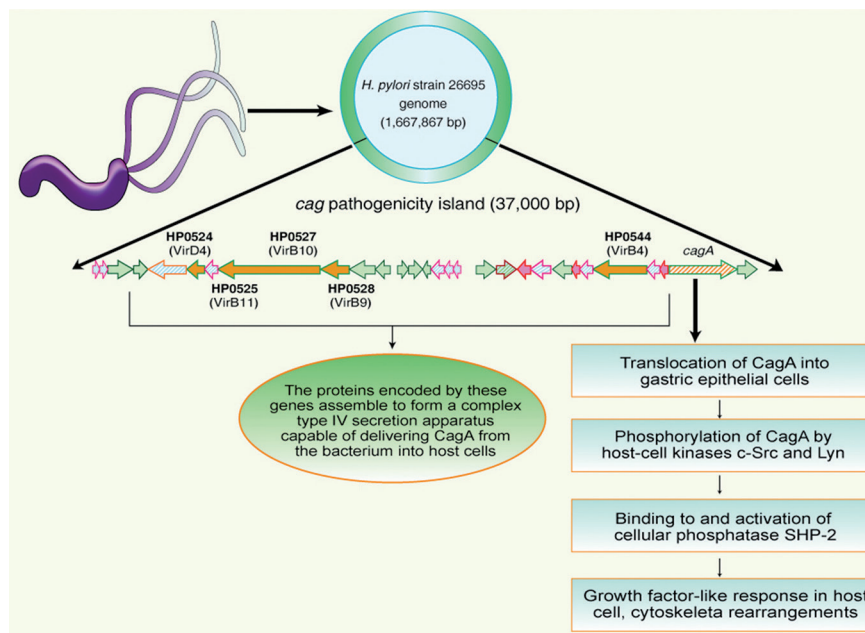


Figure 1. Structure and function of Cag pathogenicity island. This island spans for a 37,000 bp chromosomal region and includes twenty nine genes. The protein encoded by this island not only stimulate IL-8 production by gastric epithelial cells but also assist translocation of CagA into the host cell. The genes responsible for IL-8 production are indicated by solid arrows. And, the blue lined arrows indicates genes which form a type IV secretion apparatus that make a channel for CagA trafficking. Additionally, orange lines indicates other virulence genes involved in pathogenesis.

H. pylori strains are varies with OMPs expression and adhesins properties. One of the outer membrane protein OipA (HopH) also express differentially due to phase variation, which result in strain specific signaling in gastric epithelial cells.²² Moreover multiple factor e.g. CagA+, s1-VacA+,

BabA+ are found to be lofty interactive with the host, whereas strains that lacking these factors are under interactive (Figure 2). These CagA, s1-VacA, and BabA positive strains increases gastric mucosal inflammation and gastric epithelial injury, compared with other strains.^{23,24,25}

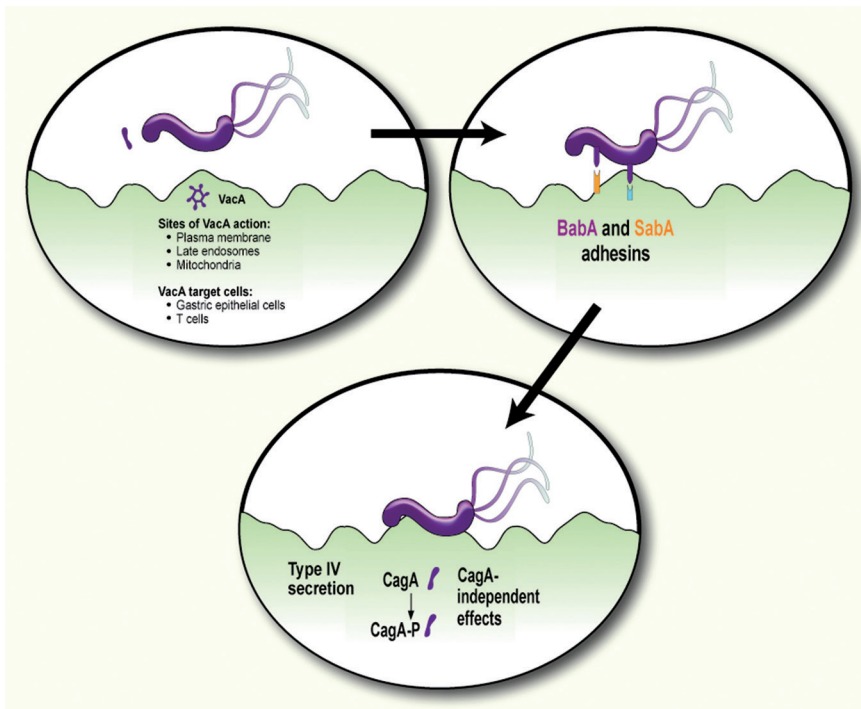


Figure 2. Host-pathogen interaction pattern.²¹ Most of the *H. Pylori* initially localize within the gastric mucus layer but do not directly adhere to gastric epithelial. Different factors especially VacA, released by these non-adherent bacteria, causes alterations in gastric epithelial cells. Besides, BabA and SabA of *H. Pylori* surface mediates crucial interaction with gastric epithelial cells. The cag pathogenicity island of adherent pathogen encode proteins that amalgamate into a secretion apparatus by which CagA factor translocates into the gastric epithelial cells. Inside the gastric cells CagA protein undergoes phosphorylation by host kinases. Both the state of CagA either phosphorylated and non-phosphorylated can trigger extensive cellular alterations.

H. pylori and host microRNA interaction

Small noncoding RNA known as microRNAs that usually bind 3' un-translated region (UTR) of mRNAs and regulate gene expression by post-transcriptional gene silencing. Variable miRNAs expression frequently found in cancer cells that reveal their functions as either oncogenes or tumor suppressors. In *H. pylori*-infected host cells only one miRNA has been identified as increased expression level.²⁶ To combat invading pathogens and their secreted effectors host cell utilize Toll-like receptors (TLR) and Nod-like receptors (NLR).²⁷ Inside the host cell these receptors subsequently trigger a set of adaptor proteins and transcription factors that finally stimulate host innate immunity through the activation of nuclear factor- κ B (NF- κ B) and activator protein 1 (AP-1) pathways. Since indecent NF- κ B activation trans-activates several target genes harbouring inflammatory, NF- κ B would be a prospective molecular connection between inflammation and cancer.²⁸

A number of microRNA have been identified as direct and indirect regulator of apoptosis. Among them miR-155 is one of the foremost target of TLR pathway that activated during microbial infection in myeloid cells. Besides, different factors of TLR signaling mechanism including FADD, RIP, and IKK are also down regulated by miR-155.²⁹

Conversely, with the up-regulation of miR-155, another microRNA miR-125b marked at a decreased level. Low expression level of miR-125b allows TNF- α synthesis in the way of TLR stimulation that has a role in apoptosis.^{30,31} Under noninfectious condition, miR-125b generally repress TNF- α synthesis that ultimately suppress the synthesis of other pro-inflammatory cytokines including TNF- α itself.³²

miR-146a is also stimulated by NF- κ B via TLR2, 4, 5 signaling pathways. In presence of bacterial component, TLR-signaling adaptators IRAK1 and TRAF6 include this miRNA into a negative-feedback loop to control TLR signaling.^{34,35}

MiR-21 is also induced by activator protein AP-1 which the signal transducer of transcription factor (STAT3).^{36,37} *H. pylori* infection induce NF- κ B and IL-6 secretion in the gastric epithelial, which first elicit AP-1 followed by the activation of STAT3 pathway.³⁸ Another study have showed that miR-21 up-regulation promote cell proliferation, migration and inhibition of apoptosis in the cell line.³⁹

MiR-27 an oncogenic miRNA also identified in gastric adeno-carcinoma cell in which it targets prohibitin a tumor suppressor. Prohibitin is an evolutionary conserved and ubiquitous protein of cell-cycle that interact with pRb and control cell cycle.^{40,41}

Role of E2F1 in the up-regulation of micro RNAs, the transition of G1/S stage of cell cycle

depends on E2F1 transcriptional factor which sequestered by the unphosphorylated retinoblastoma protein pRb in resting cell. Although selfly activated E2F1 trans activates a cluster of intronic miRNAs e.g. miR-106b, miR-93 and miR-25 mounted in the *Mcm7* gene which accumulate in gastric primary tumors and gastric epithelial cell lines.^{42,43} Conversely, miR-106b and miR-93 inhibit E2F1 self-activation and suppress E2F1 expression level through a negative-feedback loop.⁴⁴ In addition to E2F1, p21/CIP1/WAF1 also repressed by miR-106b, miR-93 and miR-25 that leads to the decreased response of gastric cells to TGF β .

miR-106b-25 cluster, locates at chromosome 13q31 and consists genes of miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92-1. The cluster of miR-17-92 also identified as the partner of oncogenic potential. In several carcinomas cell like B cell, lung, colon and pancreas miR-17-92 expres-

sion level found elevated.^{45,46} Moreover, gastric cell also tested as higher expression level of miR-17-92 during its cancerous state.⁴⁷ In addition to the miR-106b-25 cluster, the miR-221-222 cluster has also increased highly in gastric tumor cell.⁴⁸

microRNA regulation in gastric cancer: Differential expression pattern of miRNAs modulate apoptosis by altering the expression of Bcl-2 family members in gastric tumor (Figure 3). For instance, TGF β induce apoptosis in gastric cancer cells through the sequential activation of RUNX3, FoxO3a/ FKHR L1 and finally pro-apoptotic protein Bim. In this regard, microRNA miR-106b and miR-93 inhibit Bim expression and thus impair TGF β -induced apoptosis in gastric cancer cells. Moreover, several miRNAs, including miR-15b, miR-16, miR-34 and miR-181b, have also been shown their interaction with anti-apoptotic protein Bcl-2 and instigate apoptosis.⁴⁹

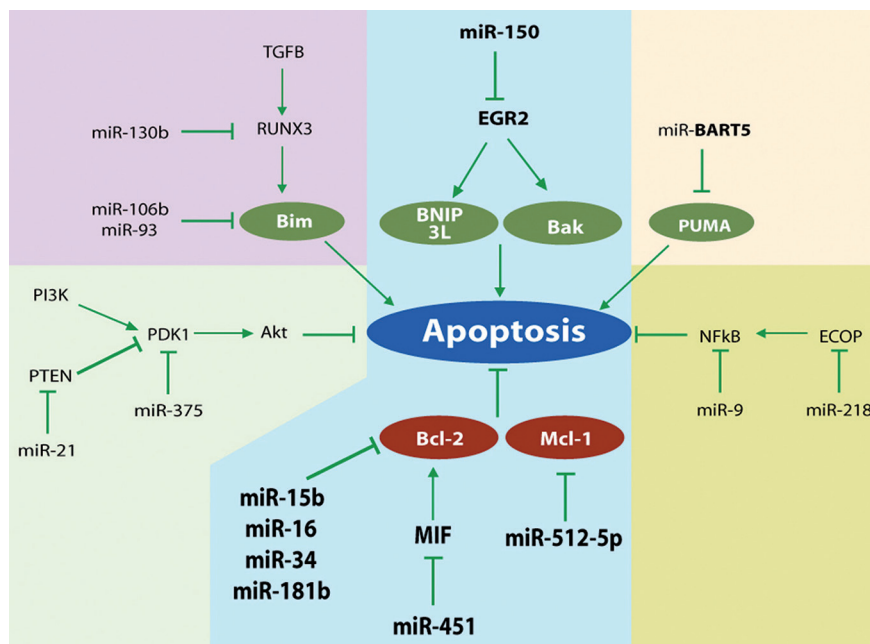


Figure 3. Co-regulation of miRNA and apoptosis in gastric cancer.⁴⁹ Multiple number of miRNAs have been shown to directly or indirectly regulate proapoptotic and anti-apoptotic members of Bcl-2 family. These miRNAs also regulate the PI3k/Akt and NF-kB signaling pathways and exert apoptotic signal that alter the steady state of gastric cancer cells.

Pathogenesis of *H. pylori* infection

Colonization of *H. pylori*: The ability of *H. pylori* to inhabit the human stomach can be attributed to the assembly of specific toxins or other effectors (Figure 2). One of the bacterial products urease hydrolyzes urea to yield ammonium ions and thereby *H. Pylori* altered to acid resistance.⁵⁰ The motility of flagella enable *H. pylori* to penetrate the gastric mucus layer.

The outer membrane proteins BabA, SabA, AlpA, AlpB, and HopZ, can adhere *H. pylori* to gastric epithelial cells. Adherence of *H. pylori* to gastric epithelial cells activate a number of signaling pathways⁵¹ and permits toxins efficient delivery or other effectors into the cells. A number of experiment in an animal model have showed that *H. Pylori* adherence to epithelial cells influences gastric mucosal inflammation, production of auto-antibodies, and parietal cell loss.⁵²

Early Immunomodulation: Altered form of *H. Pylori* lipid A makes its surface LPS less immune sensitive than other negative strain bacterial LPSs. *H. pylori* flagella are poorly recognized by TLR5 due to the modifications of TLR specific surface antigen.⁵³ LPS O antigens are abundance on numerous *H. pylori* strains which structurally similar to human Lewis blood group antigen.⁵⁴ That's why, inside the host cell *H. pylori* LPS antigen treated as native.⁵⁵ Besides, modified form of cholesterol on *H. pylori* surface and host plasminogen coating also could resemble as antigenic disguise.⁵⁶ Moreover, several factors also released due to intervention of *H. pylori* in gastric epithelia that also excites host immune cell. Likewise, *H. pylori* secreted (VacA) factor stimulate CD4+T cells, inhibiting the transcription factor of T cells proliferation. VacA also restrict B cell antigen presentation and hinder CD8+T, macrophages and mast cells regular function.⁵⁷

There are two other protein arginase and glutamyl transferase expressed in *H. pylori* also alter T cells function. *H. pylori* arginase directly react on macrophages and down-regulates its nitric oxide synthesis.⁵⁸ *H. pylori* generally target host immune cells and strongly causes their down-regulation and thus lengthen its persistence.⁵⁹

Acute inflammation: The gastric epithelium of *H. Pylori* infected persons has enhanced levels of IL-1 β , IL-2, IL-6, IL-8, and TNF α . Especially, IL8 act as a potent neutrophil activator expressed by gastric epithelial cells, play central role in acute inflammation.^{60,61} It has been validated experimentally that *H. pylori* strains carrying the cag-PAI, provoke a far stronger IL-8 response than cag-negative strains. And the central partners of this IL-8 response are nuclear factor kB (NFkB) and transcription factor activator protein 1 (AP-1).⁶² Moreover another surface protein (150kD) of *H. pylori* activates neutrophil may stimulate phagocytes, though its relation to clinical outcome is still remains indistinct.⁶³

Persistent inflammation leads to cancer: All sorts of chronic injury and irritation by infection or no-infection, initiate an inflammatory response. During *H. pylori* infection CagA, get entry into the epithelial cell, induces proliferation and motility signals, as well as production of cytokines. Several mechanisms by which CagA induces the transformational changes in a host cell have been identified, such as its interaction with SHP-2 protein and reaction with cytoplasmic tyrosine phosphatase.⁶⁴

At the injection of CagA into the cell, primarily it phosphorylated by Src family kinases and secondly

go interaction with the SHP-2.⁶⁵ Since SHP-2 play an crucial role in regular transduction events, CagA hinder cellular functions by deregulation of SHP-2, inducing cytoskeletal rearrangements, proliferation and increased motility of gastric epithelial cells. Thus CagA-positive *H. pylori* infection up-regulates COX-2 expression in gastric mucosa and finally lead to cancer. In resting cell COX-2 is usually absence but at sites of inflammation it becomes abundant and in gastric carcinomas it goes for over expression.^{66,67} This abnormal expression of COX-2 leads to an enhanced genesis of prostaglandins, such as PGE2. Finally, This COX-2 induced prostaglandin pathway accelerate carcinogenesis (Figure 4) by modulating cell proliferation, inhibiting apoptosis and by increasing invasiveness of malignant cells.

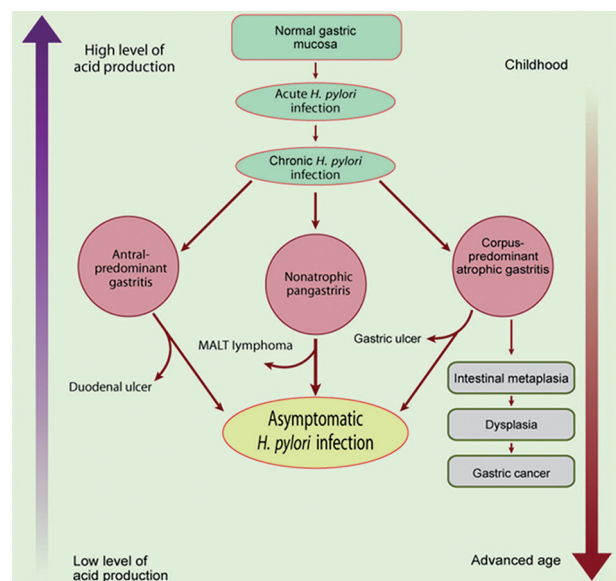


Figure 4. Overview of *Helicobacter pylori* Pathogenesis. Normally people acquire *H. pylori* in childhood. At acute phase it causes transient hypochlorhydria which is rarely traced by the patients. While colonize persistently in gastric mucosa, it gradually undergoes chronic gastritis without notifying any virtual symptoms. Along with the increment of patient ages, acid production raises steadily and leads to antral-predominant gastritis, which is the major cause of duodenal ulcers. On the other hand, patients with lower acid output for a long period are more likely to have gastritis in the stomach, which also leads them to gastric ulcer and can initiate a number of sequential events that, in rare cases, could initiate gastric carcinoma. Another rare complication of *H. pylori* infection is the formation of mucosa-associated lymphoid tissue (MALT) which may finally leads to malignant lymphoma.

CONCLUSION

H. pylori is one of the most common bacterial infections in human, colonizing in the stomach and persist life time in host. Although the human host mounts a vigorous innate and adaptive immune response against the bacterium, but it escapes and evades host responses by a variety of strategy, leading to persistent colonization and chronic active inflammation. Clinical complications of *H. pylori* colonization mainly gastric cancer are therefore likely to represent imbalances in gastric homeostasis that are unexpected for both microbe and host. Extensive experiment based on interaction between host immune cell and *H. Pylori* could unveil the hidden mechanism of its persistence colonization which could be a new beacon for therapeutic advances.

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