The Virulence of *Helicobacter pylori* Through CagA on Gastric Mucosa: A Review

Shida Hussain[1], Zhang Li[2], Xiaolan Lu[2], Hamad Haider Khan[3], Shi bingyin[3], Faiz Ullah Khan[4,5,6,8,*]
Zakir Khan[7], Beenish Ashfaq[8], Inayat Ur Rehman[9,10]

[1]Registrar, Department of Nephrology Northwest General Hospital Hayatabad Peshawar, Pakistan, PAKISTAN.
[2]Departmet of Gastroenterology Second affiliated hospital, Xi'an Jiaotong University, School of medicine, Shaanxi 710061, Xi'an, , CHINA.
[3]Department of Clinical Endocrinology Xi'an Jiaotong University, First Affiliated Hospital, Xi'an, Shanxi, CHINA.
[4]Department of Pharmacy Administration and Clinical Pharmacy, School of Pharmacy, Health Science Centre, Xi'an Jiaotong University, CHINA.
[5]Center for Drug Safety and Policy Research, Xi'an Jiaotong University, Xi'an, CHINA.
[6]The Global Health Institute, Xi'an Jiaotong University, Xi'an, China; d Shaanxi Centre for Health Reform and Development Research, Xi'an, CHINA.
[7]Department of Pharmacology, Institute of Health Sciences, Cukurova University, Adana,TURKEY.
[8]Department of Pharmacy Quaid-i-Azam University Islamabad, PAKISTAN.
[9]School of Pharmacy, Monash University Malaysia Jalan Lagoon Selantan Bandar Sunway, MALAYSIA.
[10]Department of Pharmacy, Abdul Wali Khan University, Mardan, PAKISTAN.

Abstract

Helicobacter pylori (*H. pylori*), is the most common infectious bacterium of the Human stomach. In early childhood, colonization of *H. pylori* in stomach releases different pathogenic factors which interfere with functions of human cells. Combination of two main genetic pathogenic factors CagA and VacA play important role in causing severity of *H. pylori* infection. In *H. pylori* infected patients, CagA and CagPAI positive strains have positive association in development of peptic ulcer disease and gastric cancers.50-70% of *H. pylori* produces CagA gene that is multifunction toxin. By adapting different mechanisms CagA define its role in oncogenic signaling cascades, in tumor suppressor pathway and in inflammation. CagA gene is strongly associated with VacA cytotoxic function and the strains, ultimately responsible for epithelial destruction of stomach and leading towards cancer. Review provides, CagA has important role in the virulence of *H.pylori* infection. Different Peptic ulcer diseases and gastric cancers are caused by the presence of CagA gene.

Key words: *H. pylori*, CagA gene, VacA gene, Gastric cancers, Peptic ulcer disease.

INTRODUCTION

Helicobacter pylori

Helicobacter pylori (H. pylori), a slow-growing, helical shaped, gram-negative, flagellated and microaerophilic bacillus, is the most common infectious bacterium of the human stomach, infecting more than 50% of the human population worldwide.[1] Developing countries have higher contamination with *H. pylori* with a prevalence of greater than 80%. This bacillus is 2.5 to 5 µm long and 0.5 to 1.0 µm wide, with 4 to 6 flagella for its movement and can live in such an environment that no other bacteria are able to survive.[2] Two Australian scientists Warren and Marshall, awarded Nobel Prize in 2005, discovered the correlation between colonization of *H. pylori* in the stomach and Peptic Ulcer Disease (PUD). The infection usually takes place in early childhood and adapts to human colonization by producing pathogenic factors such as urease, catalase, cytotoxin and Lipopolysaccharide (LPS), which directly interfere with the functions of the host cell and sustains throughout life in the stomach causing disease mainly in adults if not eradicated by therapy.[3] Bacterial, environmental and genetic factors affect the severity of *H. pylori* infection, which may involve the combination of these factors.[4,5]

The related mechanism has not been completely revealed yet; however, a combination of different pathogenic factors, of which two main factors are CagA and VacA, may play a role. Due to the gastric colonization, the persistent infections can be associated with acute or chronic inflammation and most of them are asymptomatic, however the risk of acquiring site-specific disease is augmented.[6,7] Its colonization does not produce any symptoms itself and the symptoms are established in less than 20% of all infected individuals due to their infection.[8] Many studies show that persistent inflammation and abnormal epithelial proliferation during *H. pylori* infection are main factors that cause *H. pylori*-associated gastric diseases such as gastritis, PUD (90% of duodenal ulcers and 80% of gastric ulcers),[9,10] gastric adenocarcinoma and lymphoma (Mucosa-associated lymphoid tissue).[11,12] In 1994, *H. pylori* is stated as a group 1 human carcinogen by the World Health Organization and the International Agency for Research because of its effectively crucial roles in the development of both intestinal-type (About 90% of cases) and diffuse-type (About 32% of cases) gastric adenocarcinoma.[13,14] In *H. pylori* infected patients, studies showed that there is a positive association between *H. pylori* CagA-positive strains and the development of peptic ulcer disease and Gastric cancer (GC).[15,16] Patients with duodenal ulcers have reduced risk

of arising gastric cancer comparing to those with gastric ulcers.[17] Further studies have showed that many extragastric diseases, including Idiopathic Thrombocytopenic Purpura (ITP),[18,19] Iron Deficiency Anemia (IDA),[20,21] fatal cardiovascular events,[22-25] autoimmune thyroid diseases,[26] Diabetes Mellitus (DM)[27-29] and hepatobiliary diseases,[30-32] may also be caused by *H. pylori*.

Cytotoxin-associated gene A

Cytotoxin-associated gene A (CagA), a 120 to 145 KDa protein encoded by CagA gene (One of 32 genes of CagPAI), is a multifunctional toxin secreted by *H. pylori* and is translocated into the host target cells *via* Type 4 Secretion System (T4SS) during *H. pylori* infection.[33] Approximately 50%-70% of *H. pylori* strains have CagA gene, whose prevalence is approximately 90% in certain Asian countries. This multifunctional toxin is involved in the distraction of the apical-basolateral polarity of epithelial cell and cell tethering and is also involved in the stimulation of cell proliferation, migration and cell morphological variations.[34,35] Through these mechanisms, CagA induces epithelial to mesenchymal transition and cancer cell invasion. Interestingly, besides induction into gastric cells, CagA can also be inducted into B-lymphocytes[36] and into dentritic cells of murines and humans that dampens the immune response by lessening proinflammatory cytokines expression and augmenting suppressive cytokines production.[37,38] This shows double pro- and anti-inflammatory function of CagA during *H. pylori* infection reliant on the cellular milieu.

This bacterial protein contains a distinctive tertiary structure comprising of a solid N-terminal region (70% of the whole CagA) and an instinctively disordered C-terminal tail (30% of the whole CagA) that has the EPIYA segment and the CagA-multimerization (CM) motifs.[39,40] The structural diversity in C-terminal tail gives rise to the distinction in the size of the CagA.[41] A square plate-like shape is predicted by the N-terminal core, which contains three distinct domains 1-3. Domain 1 is mobile and flexible and is the most N-terminal domain having a minor interacting surface area with domain 2 but not domain 3. Domain 2 has a protease-resistant structural CagA core and also has a large antiparallel b sheet for binding of CagA to b1- integrin of the host membrane for its translocation. Domain 3 also consists of a protease-resistant structural CagA core. A lariat loop is produced *via* looping back of the disordered C-terminal tail onto domain 3. CagA gene, a polymorphic gene, displays various repeated sequences situated in its 3' region and each repeated region has Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, consisting of site for tyrosine phosphorylation.[42] The disordered C-terminal region has four EPIYA motifs, designated as EPIYA –A, -B, -C and –D on the basis of amino acid sequences fringing the EPIYA motifs,[43,44] with variations in geographical distribution and also in degrees of phosphorylation.[16,44] The phosphorylation of EPIYA-A and EPIYA-B is lesser than that of EPIYA-C and these motifs are found throughout the world. Only strains from Western countries have EPIYA-C, which is a marker of the risk of gastric adenocarcinoma. Therefore, the Western strains of *H. pylori* contain EPIYA-repeat region having the sequence of EPIYA –A, -B and -C motifs (A-B-C type CagA).[45,46] East Asian strains have EPIYA-D, which provokes more release of interleukin-8 (IL-8) from the host epithelial cells of the stomach than the other EPIYA motifs and these motifs have greater extent of phosphorylation than the other EPIYA motifs.[47] Thus, the East Asian strains of *H. pylori* contain EPIYA-repeat region having the sequence of EPIYA –A, -B and –D motifs (A-B_D type CagA).[41] Following the delivery into the gastric epithelial cells, CagA deregulates multiple signaling pathways through interaction with many human proteins in both phosphorylation-dependent and phosphorylation-independent manners.[48,49] Studies showed that CagA behaves as a promiscuous scaffold or hub protein because of its intramolecular interactions and the disturbance of various host proteins instantaneously. After translocation, CagA is bound to the inner side of cell membrane. This mechanism of interaction between CagA and host cell membrane is complicated and reliant on the cellular milieu. This mechanism concerns two distinct CagA regions, the basic patch and the EPIYA motifs.[39] The basic patch, a cluster of basic residues on the surface of CagA domain 2, adheres like Velcro to phosphatidylserine, which is an acidic phospholipid precisely determined to the inner side of cell membrane and, in polarized host cell, this CagA-phosphatidylserine interaction has a crucial function in the binding of CagA to the cell membrane.[50] The EPIYA motifs are important for the connection of CagA with cell membrane in nonpolarized epithelial cells. Abl and Src kinases of the host carry out the phosphorylation of CagA at EPIYA regions.[51] Src kinases preferably at EPIYA-C and/or EPIYA-D regulate early phosphorylation and Abl kinases at any site regulate later phosphorylation during *H. pylori* infection.[48,52] Evidences have shown that CagA acts as the only known bacterial oncoprotein in human population.[53] On the basis of CagPAI-encoding, clinical isolated *H. pylori* strains are frequently subdivided into two kinds of strains, the CagA-positive strains and CagA-negative strains. About 6:4 is the infection ratio between CagA-positive and CagA-negative strains worldwide but East Asian countries are exception because of nearly all infections with CagA-positive strains of *H. pylori*. Patients with CagA-positive strains of *H. pylori* in Western countries have a higher risk of developing gastric adenocarcinoma than those with CagA-negative strains.[54,55] Many evidences suggest that the CagA-positive strain is the chief *H. pylori* strain that leads to carcinogenesis in gastric epithelial cells.[55,56] Nevertheless, many strains of *H. pylori* in East Asia contain CagA gene, which is regardless of the disease.[57] Furthermore, although situated in distinctive genomic loci, there has been established a vital correlation between VacA s1 (Either m1 or m2) stains and CagA positive strains of *H. pylori*.[58] VacA, a 140 KDa cytotoxin present in all strains, is produced by the bacteria and latter transformed into active form and finally inducted into host target cells.[59] CagA gene is strongly associated with the VacA cytotoxic function and the strains, which contain the arrangement of these genes, are supposed to be the most virulent strains of *H. pylori*,[60] leading to more severe epithelial destruction in the stomach and the occurrence of the most severe stomach diseases.[13,44,61]

Cag Pathogenicity Island

Cag Pathogenicity Island (CagPAI) is a 40 Kb DNA fragment having approximately 32 genes that encode for the various constituents of type 4 secretion systems which are crucially important for cag translocation and some of them furthermore play a crucial part in inflammatory reactions in the target cells during pathogenesis.[62] Besides several virulence determinants of *H. pylori*, CagPAI plays a crucial role in the development of PUD and GC.[16] CagPAI is supposed to be entered through horizontal transfer from an unidentified organism into the *H. pylori* genome. It can also encode one of the most copious *H. pylori* proteins, CagA, which is delivered into various cells including gastric and non-gastric during infection. The existence of CagPAI and CagA is very much important in the pathogenesis during *H. pylori* infection. According to a novel insertion series, CagPAI can be alienated into two sections cag1 region and cag2 region.[63] CagPAI occurs in about 60% to 70% of *H. pylori* strains in Western world and almost 100% in East Asian world.[62] Cag-positive strains (Containing CagPAI) occur near or adherent to epithelial cells in stomach and cag-negative strains (lacking CagPAI) occur mainly in gastric mucosal layer.[55] Cag-positive strains raise the risk for more severe inflammation, dysplastic changes and carcinogenesis than cag-negative strains of *H. pylori* in the host target cells.[44,55]

Furthermore, studies have shown that CagPAI seems to be intricate in the stimulation of IL-8 production and activation of NF- ϰB in gastric epithelial cells.[64,65] This is also ominously explained in the mucosa of stomach of

Mongolian gerbils infected with cag-positive *H. pylori* strains;[66] however, some studies suggest that this IL-8 production and activation of NF-ϰB does not depend on CagA but rather depends on T4SS encoded by *cag*PAI.[67] So consequently, the CagPAI existence leads to proinflammatory reactions in the target cells. Though many virulence factors are displayed during infection, but CAGPAI seems to be the most significant factor that is involved in the occurrence of peptic ulcer disease and gastric cancer.

Adaptation of *H. pylori* in the Gastric Niche
Gastric colonization of H. pylori

H. pylori have a very firm tropism in the tissue of the human stomach. This bacterium gets access to the human gastric mucosa and colonizes only in the other body's areas where gastric metaplasia occurs. *H. pylori* specially settle the gastric niche and can produce inflammations as well as malignancies.[11,68] Great progress has been achieved in the understanding of factors related to pathogenicity of *H. pylori* and their interactions with the constituents of gastric epithelium. These factors are membrane-associated, secreted, or delivered to the cytoplasm of the gastric epithelial cells. *H. pylori* also contain metabolic factors that make the bacterium able to change the gastric niche of the host for its own purpose.

In 1997, the genome of *H. pylori* was fully sequenced,[62] which is as small as a size of one-third of the genome of *E. coli*.[69] This accelerates and facilitates further studies in the research field on *H. pylori* infection and its colonization in human gastric epithelial cells. *H. Pylori* lives and adapts in the gastric epithelium because of its persistent and close association with the gastric niche throughout life. *H. pylori* genome has high diversity,[70] which can crucially contribute in the adaptation and persistence of *H. pylori* in the host gastric epithelial cell, as well as in the infection with *H. pylori*. The anatomical distribution of *H. pylori* infection and its clinical outcome are impacted significantly by the genetic factors[54] and the risk for gastric cancer development is considerably augmented by the polymorphisms in many genes.[71] The risk of gastric cancer development is two-folds to three-folds greater in individuals having the pro-inflammatory polymorphism of the IL-1β and IL-1 receptor antagonist genes than those individuals who have less pro-inflammatory genotypic activity.[72] Similarly, increased risk of gastric cancer development is also related with polymorphism in the genes that control the tumor necrosis factor-α (TNF-α) and the IL-16.[71,73] Conclusively, the variation in localities and structural alterations during *H. pylori* infection appear to be vital for lessening host immune system and help in the adaptation of *H. pylori* in gastric niche, showing that *H. pylori* can manipulate many processes of epithelial cell and to deregulating multiple signaling pathways. This results in bacterial attachment to the cell, introduction of pro-inflammatory reactions by releasing cytokine/chemokine, induction of apoptosis, proliferation and an augmented mitogenic response, which eventually result in sustained colonization, intense inflammation, distraction of gastric epithelial barrier function and gastric adenocarcinoma.

H. pylori Colonization determinants
Adherence determinants on signaling cascades

The first step in pathogenicity of *H. pylori* is supposed to be its adhesion to the gastric epithelial cells. This is carried out by the large group of outer membrane proteins,[74-76] which consists of some adhesins, such as Blood-group-antigen-binding adhesion (BabA), Sialic acid binding adhesion (SabA), adherence associated lipoprotein A and B (AlpA/B) and Outer inflammatory protein A (OipA), which facilitate the attachment of *H. pylori* to the gastric epithelial cell membrane during pathogenesis[77] and other factors, such as urease, catalase, cytotoxin, lipopolysaccharide and flagellin, which are capable

of eliciting inflammatory reactions in gastric epithelium. The adhesion of *H. pylori* to the gastric epithelial cell is extremely necessary for the pathogenesis of *H. pylori*,[78] but there are sparse evidences of showing that these adhesins have direct effect on signaling cascades. This shows that these adherence factors may facilitate a tight communication among *H. pylori* and the gastric epithelial cell, possibly providing the approach for the interaction of other bacterial factors with the target receptors of the host cell. Several adherence determinants acting on signaling cascades have been recognized yet which are capable of altering the pathways of signal transduction and carrying out other bacterial virulence factors to interact with the host gastric epithelial cell membrane, which are normally not capable of interacting and altering signal transduction pathways.

Other putative adherence determinants

There are many other putative adherence determinants that play a role in the adhesion of *H. pylori* to the host target cell. HopZ, a phase-variable protein, has been implicated in *H. pylori* attachment during pathogenesis[79] and current studies disclosed that it has a role in the initial phase of colonization. Upon re-isolation from a healthy volunteer confronted with HopZ "off" status presented a robust *in vivo* selection for the HopZ "on" *H. pylori*.[80] In another study performed by Snelling and co-workers, HorB was proposed to have a role in *H. pylori* adhesion.[81] Another putative adherence determinant is HopQ, which has an adhesion-related function on the host target cell. In a subdivision of *H. pylori* strains, the deletion of HopQ augmented the attachment of *H. pylori* to AGS cells and guided them to a phenotype of increased adherence and consequently to the augmented phosphorylation of CagA, without any effect on IL-8 production.[82] Therefore, HopQ extensively led to lessening of CagA instillation into the host gastric epithelial cells in co-infection experiments.[83]

Initiative determinants in pathogenesis

Many secreted determinants are highly capable of causing virulence in *H. pylori* infections deprived of any interaction or attachment to the host target cells. Several secreted or extracellular determinants have been recognized in the *H. pylori* secretome analyses.[84,85] Besides uncharacterization of many *H. pylori* extracellular proteins, there is progressively improvement in the knowledge about γ-glutamyl transpeptidase (GGT), *H. pylori* Neutrophil-activating protein (HN-NAP), urease, Vacuolating cytotoxin A (VacA), Cag pathogenicity-associated island (PAI) and a High temperature requirement A (HtrA). For instance, GGT has been recognized in the *H. pylori* soluble fraction and it has been revealed to augment *H. pylori* colonization in mice.[86] Fascinatingly, apoptosis and cell cycle arrest can be induced by recombinant GGT in AGS cells,[87,88] but this mechanism has not been clarified yet. HP-NAP, a chemotactive factor of *H. pylori* attracting and activating neutrophils,[89] has no obvious role in interaction of *H. pylori* with gastric cells. Moreover, urease, VacA, CagA and HtrA have several direct effects on host target cells, including introduction of apoptosis and waning the internal structure of intracellular bindings.

Peptidoglycans

Besides the roles in making the cellular structure of *H. pylori* and supporting *H. pylori* colonization, peptidoglycans are also the factors of *H. pylori* that are delivered to the host target cells *via* type 4 secreting system and Outer Membrane Vesicles (OMV).[90,91] By interacting with nucleotide-binding oligomerization domain 1 (Nod 1), peptidoglycan results in the triggering of proinflammatory IL-8, MIP-2 and β-defensin-2 production *via* activation of NF-ϰB, p38 and Erk signaling in the target cells.[44,92,93] Moreover, Interferon-1 (IFN-1) production is regulated by the activation of

peptidoglycan-Nod1 signaling, which can mimic TH1 cell differentiation.[94] Furthermore, a reduction in mucosal cytokine reaction was discovered in NOD1 lacking mice that were infected with CagA-positive strains of *H. pylori*,[95,96] showing that peptidoglycan-NOD1 signaling is vital in the immune reactions concerning *H. pylori*.[97,98] However, the preference of peptidoglycan for delivery into the target cell through T4SS-dependent manner or transmembrane delivery through Outer membrane vesicles (OMVs) before NF-*x*B stimulation still requires further evaluation.[90]

In addition, the transfer of peptidoglycan into the target cells causes possible carcinogenetic reactions like cell immigration and PI3K stimulation. A lytic transglycosylase, encoded by slt gene of *H. pylori*, is necessary for the production and turnover of peptidoglycan,[99] thus restraining the amount of peptidoglycan transferring components because inactivation of slt gene results in inhibition of *H. pylori* causing cell immigration and PI3K stimulation.[100] HP0310 gene of *H. pylori* is necessary for peptidoglycan production and causes deacetylation of peptidoglycan that is essential for adaptation and colonization of *H. pylori* in gastric epithelial cells.[101,102] Deficit of HP0310 gene results in lessened peptidoglycan synthesis and intensified delivery of other main Cag T4SS molecules, CagA, implying an association between virulence factors of *H. pylori*.[103]

H. pylori translocation and type 4 secretion system

Many evidences demonstrated that after *H. pylori* colonization in the gastric epithelial cells, the delivery of CagA into the host target cells is arbitrated by T4SS, by communicating with the cell membrane of the gastric epithelial cells.[104,105] Thought the precise mechanism is undetermined, it appears to be elicited by the collaboration between T4SS pilus, CagL and α5β1 receptor molecule and also needs the collaboration between CagA and Phosphatidylserine (PS) situated at the exterior leaflet of host cell membrane.[50,106] Many bacterial virulence determinant proteins are transferred into the target cells *via* Type 4 secretion system (T4SS) and are necessary for CagA delivary like CagT.[107] CagE is a basic structural and functional constituent of T4SS and its inactivation abolishes *H. pylori* protein transportation into the target cells. CagL, a well-preserved *H. pylori* determinant protein and consists of arginine-glycine-aspartate motif that allows the attachment of *H. pylori* to α5- β1 receptors on gastric cell membrane, is responsible for the production of tip of type 4 secretion system pilus, helping the CagA delivery into the host target cells and activating FAK and Src in the host cells and can also attach to integrin and fibronectin with unknown effects.[108] CagI, not necessary for transportation of CagA but is a vital component of T4SS, is manipulated by the appearance of other *cag*PAI products, showing that their induction needs partial gathering of T4SS.[40,109,110] CagM proteins are also intricate in CagA delivery into the target cells, but proteins having deficient mutants show a little contribution in CagA delivery during infection.[111] Recent studies suggest that CagA, CagI and CagY proteins can attach to β-1 component of target cell membrane and cause alteration in heterodimers, letting transportation of bacterial molecules.[112,113] All together, these outcomes suggest that for CagA delivery into the target cells, which depends on energy-associated target cells mechanisms, *H. pylori* manipulates the cell membrane exterior molecules, such as integrin and PS. CagA delivery also requires polymerization of actin and cholesterol of cell membrane.

After delivery of CagA into the host target cells, CagA communicate with cellular proteins and causing distraction of several signaling cascades in host cells. This results in "Hummingbird phenotype", which is an elongation caused by the establishment of pseudopodia and stress fibers from disruption of the cell-to-cell binding in gastric epithelium.[114-116]

H. pylori gastric mucosal penetration

A secreted mucosal layer shelters the whole surface of the alimentary tract and, for colonization and entry into the target cell epithelium, *H. pylori* infiltrates the gastric mucosal layer that is about 300μm thick.[117] *H. pylori* can modify the mucosal structure that may assist in passage through viscoelastic mucosal gel coating. Thioredoxin system, which particularly lessens the disulphide bonds of mucins,[118] is capable of lessening the gel-creating quality of mucins and therefore assisting in the movement of *H. pylori via* mucosal layer of the epithelium. In the absence of urea, *H. pylori* can live in 4 to 8 pH environment, but, in the presence of urea, *H. pylori* can live in pH environment as low as 2.5.[119] Studies have shown that mucin in the stomach depends on pH, as strong acidic environment results in more gel-forming mucus than at pH more than 4.0.[120] Urea consumption of *H. pylori* augments the pH, thus causing less gel mucosal coating and quick *H. pylori* delivery *via* it.[121] The role of pH is also obvious in *H. pylori* localization, normally *H. pylori* is located near the epithelium of the stomach, but the disturbance of pH gradient in mucosal coating causes *H. pylori* scattering throughout the gastric mucosal coating and no more near the epithelium.[122] As *H. pylori* has spiral structure that also assists in manipulating the mucosal-coating entry of the bacterium, modification in helical structure of *H. pylori via* variations in peptidoglycan cross-linking appears to be assistance in *H. pylori* gastric mucosal penetration in a corkscrew-like movement. Nevertheless, some mutants are not capable of colonization *in vitro* as spiral bacteria regardless of exhibiting the same motility as those of wild-type.[123]

H. pylori translocation into the target cell

The colonization of several bacteria in the alimentary tract mostly is liable on their capability of attaining the entry to the target cells, which are ordinarily not phagocytic cells. *H. pylori*, considered to be a facultative bacterium inside the target cells,[124] is only found in the host stomach attached to the epithelium as a non-invasive manner mostly, although several studies have disclosed that *H. pylori* is actually invasive pathogen.[124-126] As we know that bacteria inside the cells are more resistant to antibiotics and humoral immunity, thus *H. pylori* entry into the target cells is very important for its colonization and triggering of serious diseases,[127] as do the other invasive bacteria.

For establishing and developing of an infectious disease, pathogen-target cell interaction and the expression of certain determinants are crucial to overwhelm the host immunity. The gastric epithelial cells have apical-basolateral structure that is predominantly preserved by tight and adherence junctions and actin cytoskeleton.[128,129] CagA attaches with renowned manipulators of cellular polarization and adhesion, such as c-Met, Par proteins, E-cadherin, p120, ZO-1, catenin, etc., in phosphorylation-independent manner.[49] Consequently, CagA may distract tight junctions and adherence junctions by directly affecting the cell-cell adhesions.[130,131] The delivery of *H. pylori* into the target cells is carried out with the help of various adherence determinants, which are discussed above. A few evidences demonstrate the direct influence of these determinants on signaling cascades, but they may have a function in the communication of *H. pylori* with host target cells during infection.

CagA as an Oncogenic Driver

Gastric cancer (GC), mostly adenocarcinomas that are classified as intestinal and diffuse types, is the fourth highly usual malignancy and the second leading cause of cancer-related deaths and accounts for approximately 10%

of the total cancer-related deaths in global population.[132] The incidence of GC largely varies on geographical distribution and, actually, greater than half of the cases are reported in East Asia. Several transgenic studies have been performed in animals and yet proved CagA as an oncogenic driver.[15,133,134] Due to persistent *H. pylori* infection, gastric epithelium goes under intestinal metaplastic changes and is supposed to be the precancerous condition of the target cells.[135] Sensitivity of the target cells to the oncogenic exposure is augmented by pathological dysplasia. CagA may also manipulate cancer-related stem cells in the stomach, as CagA can provoke EMT-like variation that shows cell reprogramming.[130,136] and CagA can express CD44v9 that helps in longevity of CagA oncogenic accomplishment.[137]

The Role of CagA in Oncogenic Signaling Cascades
The Wnt/ β-catenin signaling pathway

The Wnt/ β-catenin signaling pathway, managing embryonic growth and also tissue homeostasis in adults, plays a vital function in the disease development and β-catenin is the essential target of this pathway.[138,139] As this pathway is involved in the proliferation and differentiation of the cells, the deregulation of this pathway is extensively incriminated in various human GI cancers like CRC and GC[140,141] and is carried out by pathway constituent modification, antagonist inhibition or cross-link with other pathways. Besides Wnt/ β-catenin signaling pathway activation, the alteration in β-catenin locality and the augmentation in β-catenin nuclear assembly in AGS host target cells are carried out by *H. pylori* CagA-positive strains. These modifications are also reported in *H. pylori* colonized gerbil gastric mucosal layers and human gastric mucosal layers.[142] EPIYA repeat segments are obligatory for β-catenin locality and succeeding Wnt/ β-catenin signaling pathway stimulation by CagA in phosphorylation-independent manner.[143] In Zebrafish intestinal tract, because of the transgenic CagA expression *in vivo*, augmented abnormal target cells proliferation *via* Wnt target genes stimulation discloses a relation between CagA and Wnt/ β-catenin signaling pathway.[134] During *H. pylori* CagA-positive strains infection, CagA competitively bind to E-cadherin and distract the E-cadherin and β-catenin complex production in phosphorylation-independent manner, resulting in β-catenin assembly in cytoplasm and nucleus.[131]

PI3K/Akt signaling pathway

PI3K/Akt signaling pathway, overactive in certain cancers including GC,[144,145] is triggered by certain growth factors *via* specific receptors, like Epidermal growth factor receptor (EGFR) and c-met. Infection of *H. pylori* is an effective determinant in triggering and the downstreaming of PI3K/Akt pathway, which provokes EGFR Tyr 992 phosphorylation (*via* CagA and OipA) and EGFR transactivation.[146,147] This leads to activation of PI3K p85 and phosphorylation of Akt Thr 308 and Akt Ser 473 site. During infection of *H. pylori*, the alterations in CagA causes lessened number of activated Akt Thr 308 residue and the alterations in OipA causes lessened number of Akt Ser 473 residue.[148] Fascinatingly, in early infection, EGFR is stimulated by *H. pylori* and during persistent *H. pylori* infection, CagA disables EGFR stimulation by suppressing EGFR phosphorylation.[149] Furthermore, CRPIA of CagA, located at the C-terminal region, is intricate in the downstreaming of PI3K/Akt cascade *via* collaboration with c-met, which leads to GSKK3-β (PI3K/Akt downstream target) deactivation and subsequently stimulation of β-catenin transcription.[147,148] Interacting with B-TPM, CagA also persuades PI3K/Akt signaling cascade.[150]

CagA-PRK2 interaction and inhibition of its kinase activity

PRK2 (Protein kinase C-related kinase 2), an isoform of serine-threonine kinases and is extracted first from a human cDNA in 1994, interact directly with CagA and is supposed to be recruited to the target cell membrane. Various isoforms with different names were identified shortly after, including three human isoforms PRK1 (PRK-α), PRK2 (PRK-γ) and PRK3 (PRK-β). PRK2 has a proline-containing region that occurred in the linker of C2-like domain and C-terminal domain.[151] The substrates of PRK2 consist of Nck4,[152] Grb4, Fyn[153] and cortactin.[154] PRK2 is involved in many cellular activities like augmented cellular adhesion through regulation of apical junction and cellular interactions,[153] cell cycle control[155] and cellular movement and invasion.[156,157] These activities are carried out through PRK2 stimulation that needs the interaction between rho-GTP and ACC1 (Antiparallel-coiled coil) domain, which in turn leads to PIF (PDK1 interacting fragment) discharge and thus allowing PDK1 (Phosphoinositide-dependent kinase 1) attachment and PRK2 triggering loop phosphorylation.[158] Many evidence showed that EMT (Epithelial-mesenchymal transition) is manipulated by CagA and its activities lead to the disruption of cellular skeleton, cellular polarity and adhesions and increased cellular movement, which in turn leads to tumorigenesis.[115,136,159] Consequently, the CagA-PRK2 interaction and the inhibition of its activities appear to be involved in the development of gastric disease and may also be involved in CagA oncogenic activities.

Hh signaling pathway

The Hedgehog (Hh) signaling pathway, plays an essential part in embryonic growth, tissue homeostasis in adults and carcinogenesis and it can be over-stimulated by CagA *via* overexpression of Shh (Sonic hedgehog)[160,161] that causes chemotaxis during the infection.[162,163] The Shh is chiefly expressed in gastric parietal epithelial cells and impacts on the role of fundic glands. Schumacher *et al.* found that up-regulation of sonic hedgehog by *H. pylori* CagA is manipulated through NF-κB signaling cascade.[164]

JNK signaling pathway

The c-Jun NH2-terminal kinase (JNK) signaling pathway has both tumors suppressing role and pre-cancerous role in various organs and cells.[165,166] During *H. pylori* infection, CagA as a crucial determinant can trigger the activation of this signaling cascade.[167] This mechanism is supported by the findings of Wandler *et al.* in which the expression of CagA in transgenic Drosophila is carried out by apoptosis in target cells *via* the stimulation of c-Jun NH2-terminal kinase signaling pathway.[168]

JAK/ STAT3 signaling pathway

Deregulation of the Janus kinase (JAK)/ signal transducers and activators of transcription 3 (STAT3) signaling pathway is seen in various tumors and is associated with carcinogenesis and poor prognosis. Jackson *et al.* found that *H. pylori* cagA-positive strains can provoke more stimulation of STAT3 in the gastric epithelial cells[169] and CagA needs IL-6 and gp130 receptor for STAT3 stimulation.[170] CagA induces overexpression of bactericidal lectin and regenerating islet-derived (REG) 3γ in target cells through STAT3 stimulation cascade.[169] STAT3 phosphorylation, nuclear locality and transcriptional action in target cells are reliant on non-phosphorylated

CagA during *H. pylori* infection. Consequently, *H. pylorus is* capable of swaying host defense mechanisms and smoothing the evasion of host immunity *via* deregulation of the JAK/STAT3 signaling pathway.[169,171]

The role of CagA in tumor suppressor pathways
CagA-mediated degradation of p53

Many evidences disclosed that the two vital steps in tumorigenesis are oncogenes stimulation and tumor suppressor genes deactivation. A crucial event in the development and progression of a tumor is deactivation of p53, which is a vital tumor suppressor. Wei *et al.* found that the level of p53 rises initially and then declines promptly in *H. pylori* infected gerbil gastric epithelial cells and it rises again upon persistent infection for 12 weeks.[172] They reported that CagA persuades HDM2 (Human Double Minute 2) phosphorylation, which leads to the distraction of p53. This phosphorylation and stimulation of HDM2 can be intervened *via* Akt or ERK stimulation.[172,173] The proteosomal distraction of p53 is inhibited by p14ARF (A tumor suppressor) *via* sequestration of HDM2 and inhibition of its E3 ligase action.[174] During *H. pylori* infection, CagA-induced methylation of p14ARF leads to a decline level of p14ARF, which is insufficient for HDM2 and ARF-BP1 (A ubiquitin ligase) inhibition and then p53 degradation is carried out by HDM2 and ARF-BP1 activities.[175] ASPP2 (Apoptosis-stimulating protein of p53) is a p53-binding protein and a tumor suppressor and is conscripted by CagA during infection. Then the conscripted ASPP2 interacts with p53, which is letter distracted by proteasome, showing resistance to apoptosis.[176] Infections with CagA-positive strains of *H. pylori* demonstrate an augmented prospect of having p53 mutations, which occur in 40% to 50% of GC.[177,178] *In vitro* studies, AID (Activation-induced cytidine deaminase), which is a nucleotide mutator enzyme and is manipulated by CagA during CagA-positive strains infection, causing AID overexpression in host target cells through NF-κB stimulation and ultimately eliciting p53 mutations.[179]

CagA- mediated degradation of RUNX3

Runt-related transcription factor 3 (RUNX3) usually acts as a tumor suppressor and is deactivated by CagA in precancerous lesions of the stomach.[180] During the infection of *H. pylori* Cag-positive strains, CagA prevents the RUNX3 expression by ERK/MAPK signaling cascade.[181] CagA may also augment the methylation risk of RUNX3 during infection.[182] CagA also directs RUNX3 for distraction *via* proteasome and ubiquitination.[183]

CagA-induced tumor suppressor genes hypermethylation

Several tumor suppressor genes, including E-cadherin, RASSF1, DLC1, MGMT, RUNX3, p14 and p16, are distracted in CagA-induced hypermethyltion in *H. pylori* infected epithelial cells. Infection with *H. pylori* leads to augmented production of IL-1β and Nitric oxide (NO), which paly a crucial part in methylation induced by *H. pylori*.[184,185] Cheng *et al* identified concurrently *H. pylori*-induced hypermethylated genes in mouse and human epithelial cells of GC and disclosed that the prognosis of GC is affected by *H. pylori*-induced hypermethylation of Foxd3 (A tumor suppressor).[186] Moreover, methylation caused by *H. pylori* also arises in microRNAs (miRNAs) and distraction of these miRNAs provokes tumor formation *via* their oncogenic genes stimulation.[187] Furthermore, during infection with *H. pylori*, the number of methyletransferases (DNMTs) is augmented by persistent NF-κB transcription factor stimulation.[185,188] which in turn causes hypermethylation of several genes and also gastric mucosal microsatellite instability displayed in some GC cases. These factors are involved in hit-and-run carcinogenesis due to the direct CagA action. The elevation of DNMTs also causes CIMP expression,[189] which in turn

leads to the stimulation of epigenetic changes in cells involved in chronic inflammation.

The role of Caga in Inflammation
The NF-κB NF-κB-dependent inflammation

The NF-κB (nuclear factor κB) transcription factor acts as a crucial factor in inflammatory reactions and in inflammation-induced tumorigenesis.[190] The NF-κB inhibitor IκB communicates with NF-κB factor and results in the sequestration of NF-κB in the cytoplasm of deactivated cells. The IκB kinase (IκK) stimulation *via* infectious microorganisms and certain cytokines leads to phosphorylation of IκB and by this way provokes IκB distraction that is reliant on proteosomal activity. The lessening of IκB under specific level initiates the nuclear localization of NF-κB and ensuring the stimulation of inflammatory genes expression. Through lipopolysaccharides, peptidoglycan, or T4SS, *H. pylori* is capable of stimulating NF-κB transcription factor in the target cells of gastric epithelium as well as of immune system.[191] Various studies revealed that several certain signaling cascades, including PI3K-AKT, Ras and TRAF6-TAK1, are involved in NF-κB stimulation *via* CagA upon its delivery into the target cells.[93,147] Many evidences also disclosed the direct CagA-induced NF-κB transcription factor stimulation.[147,192] Infection with CagA-positive strains of *H. pylori* can induce CIN by PAR1 inhibition[193] that results in peculiar stimulation of NF-κB production. CagA-PAR1 interaction destabilizes microtubule system and thereby triggering IκB distraction, which in turn causes lessening number of IκB and then NF-κB stimulation. Since *H. pylori* can trigger the CagA-independent NF-κB stimulation, CagA may play a role as an amplifier of inflammation in the target cells during the infection.[99,191]

The NF- κB-independent inflammation

Several studies have shown that individuals with CagA-positive strains have more serious inflammatory reactions than those with CagA-negative strains.[54] CagA can stimulate certain inflammatory factors in target cells not depending upon NF- κB transcription factor. The C-terminal region of CagA is highly disordered,[39] which may be considered as a hazard indication by inflammatory factors. During *H. pylori* CagA-positive strains infection, IL1B gene mutations are related with augmented risk of gastric adenocarcinoma[194] and this has been proved in mice, which developed gastric adenocarcinoma following stimulation of IL-1β overexpression.[195] Consequently, CagA-induced inflammatory reactions may appear to be involved in GC occurrence through CagA-IL-1β interaction.

The role of inflammation in CagA-mediated tumorigenesis

Several studies disclosed that chronic inflammation creates such an environment for the target cells and manipulates them to undergo carcinogenesis. And the role of chronic inflammation in gastric adenocarcinoma is very prominent.[196] Ohnishi *et al.* reported that CagA-mediated tumorigenesis might be autonomous by itself because there was no obvious association of inflammation with tumorigenesis in transgenic mice.[15] However, it is certain that *H. pylori* CagA strains induce serious damage to the gastric epithelium and leads to chronic atrophic gastritis and intestinal precancerous metaplastic changes. As discussed above, CagA can trigger NF-κB transcriptional factor stimulation through several mechanisms and this factor as a promoter in inflammation is highly active upon CagA delivery into the target cells. As mentioned above, CagA can also trigger STAT3 transcriptional factor stimulation, which also acts as a promoter of inflammation during *H. pylori* infection. Whether acting through SHP2-Erk-NF-κB cascades or IL-6-JAK-STAT cascade, CagA by downstreaming

signaling cascade provokes NF-*κ*B and/or STAT3, which in turn leads to the expression of certain cytokines and anti-apoptotic factors and thus inducing tumorigenesis by inhibiting apoptosis of the target cells.[197] Recently Hayashi *et al.* found that mice without Claudin-18, which is encoded by CLDN18 gene and is the chief constituent of gastric mucosal tight junctions, revealed the distraction of the junctions and then the development of chronic atrophic gastritis and precancerous intestinal metaplastic changes.[198] They reported that there was no sign of dysplasia or tumorigenesis in the mice, which means that chronic inflammation-induced tumorigenesis needs additional determinant, like CagA, to introduce carcinoma in the target cells upon persistent infection with *H. pylori*. ROS, reactive oxygen species, is stimulated by inflammasomes during inflammation and leads to DNA disruption and thereby promoting mutations. By this mean, ROS triggers autophagy in the target cells of the stomach and the distraction of CagA. CagA can get away from this autophagy in GC stem cells by the expression of CD44v9, which counteracts ROS function by augmenting glutathione level in the target cells and thus leading to longer action of CagA as a carcinogen.

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All author contributed equally to this review.

ABBREVIATIONS

None.

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