Review Article

Helicobacter Pylori Cytotoxin-Associated Gene A (CagA) and Gastric Carcinoma

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Abstract

Gastric cancer is a highly lethal disease. Establishment of H. pylori infection as a risk factor for this malignancy help identifying persons at increased risk. Studying the association between the genotypes of H pylori virulence factors and gastric carcinoma would have clinical implication in selecting patients who would benefit from H. pylori eradication treatment, with a subsequent reduced risk of gastric cancer. Many virulence factors are involved in the pathogenicity of H. pylori, including urease, vacuolating cytotoxin (a product of the vacA gene), and the immunogenic protein cagA, encoded by the cytotoxin-associated gene A (cagA). These virulence factors interact and modulate different cellular signaling pathways to induce a proinflammatory response or alter tight junctions and cell polarity, which finally favor metastasis. The cagA gene is a virulence gene located in the cag pathogenicity island of the bacterial genome and is frequently associated with more severe clinical outcomes. The cagA proteins can increase the virulence potencies of strains, by increasing host-cell cytokine production and altering protein tyrosine phosphorylation. Many studies revealed possible association between H. pylori virulence factor cagA and its genotypes and the development of gastric carcinoma among patients, which would help in minimizing the risk of gastric carcinoma development by selecting patients who would benefit from H. pylori eradication. This review highlights how upregulation of cagA gene could be a risk factor for developing gastric carcinoma and its potential relationship on selection of patients who would benefit from H. Pylori treatment.

Keywords: Cancer, cytokine, virulence

Introduction

Over the past 50 years, incidence rates of gastric cancer have steadily declined in most countries, regardless of their background risk. Yet despite an anticipated continued reduction of approximately 2.0% per year, the future burden of gastric cancer, in numbers of cases and deaths, is expected to rise as the world's population increases. Gastric cancer (GC) is the fourth most common cancer and the third leading cause of cancer-related death worldwide. GC is a multifactorial disease, in which both environmental and genetic factors have a role in its etiology. Some factors are not modifiable as age and gender, however others are (i.e. smoking and H. pylori infection). The main etiological factor for gastric cancer is the infection with Helicobacter pylori (H. pylori), the first...
bacterium recognized as oncogenic\(^{(4)}\). The International Agency for Research on Cancer had classified \textit{H. pylori} as a group 1 carcinogen in 1994 based on a thorough review of relevant laboratory and epidemiological studies and reconfirmed this classification in 2005\(^{(5)}\). \textit{H. pylori} establishes a chronic long-lasting inflammation in the gastric mucosa that might lead to chronic exposure to reactive oxygen and reactive nitrogen species, which cause DNA damage, genetic instability, and gene mutations, eventually lead to carcinogenesis\(^{(6)}\). Longstanding gastric inflammation might also induce epigenetic changes, such as methylation of genes, which also leads to carcinogenesis.

**Helicobacter Pylori**

**Background:** Helicobacter pylori was first discovered in patients with gastritis and gastric ulcers and successfully cultured in 1982 by Dr. Barry Marshall and Dr. Robin Warren of Perth, Western Australia\(^{(7)}\). More than 50\% of the world’s population harbor \textit{H. pylori}\(^{(8)}\). The prevalence differs according to age, geography, and socioeconomic status, being higher in the developing countries but lower in the developed world\(^{(9)}\). Most likely due to high hygiene standards and widespread use of antibiotics in the developed countries\(^{(10)}\).

**Pathogenesis:** A complex interplay between bacterial, host and environmental factors determine the outcome of \textit{H. pylori} infection. Bacterial factors include: i) colonization factors, ii) factors mediating tissue injury, and iii) \textit{H. pylori} virulence factors.

**i) Colonization factors**

1. **Flagella:** \textit{H. pylori} is highly motile bacteria. It has unipolar 4-6 lophotrichous spiral shaped flagella\(^{(11)}\). They allow \textit{H. pylori} to move from the lumen of the stomach, with low pH through the mucus layer to an area with neutral pH for growth\(^{(12)}\). \textit{H. pylori}’s flagella are consisted of the flagellar filament, hook, and basal body. The flagellar filament is composed of two co-polymerized flagellins (FlaA and FlaB) encoded by genes essential for bacterial complete motility (FlaA and FlaB). The hook is consisted of FlgE that links the flagellar filament with the basal body. The basal body contains several protein structures that provide the energy source for motility\(^{(13)}\).

2. **Urease:** \textit{H. pylori} adjusts the periplasmic pH in the gastric harsh acidic environment by regulating urease activity. There are seven genes in the urease gene cluster, including catalytic subunits (ureA/B), an acid-gated urea channel (ureI), and four accessory assembly proteins (ureE-H)\(^{(9)}\). UreI (proton-gated urea channels) present in the inner membrane are closed at pH 7.0 and fully open at pH 5.0, enabling the rapid entry of urea into the bacterium\(^{(14)}\). Therefore, \textit{H. pylori} produces unusually large amounts of urea-derived ammonium\(^{(15)}\). Moreover, urease regulates \textit{H. pylori}-macrophage interactions by modulating phagosome pH and megasome formation that is essential for \textit{H. pylori} survival in macrophages\(^{(16)}\).

3. **Adherence factors:** \textit{H. pylori} possess fibrillar adhesions that prevent the organism from being shed during cell or mucosal turnover. They are located on \textit{H. pylori} surface that attach to the carbohydrate receptors of the mucosal cell\(^{(17)}\). The blood group antigen binding adhesin A (BabA) is the best-characterized adhesions (a 78-kD outer-membrane protein). It is relevant in diseases associated with \textit{H. pylori} as it influences disease severity\(^{(18)}\).

**ii) Factors mediating tissue injury**

1. **Lipopolysaccharides (LPS):** \textit{H. pylori} outer membrane integrity is maintained by phospholipids and lipopolysaccharide (LPS). They interfere with the interaction
between gastric epithelial cell and laminin, resulting in loss of mucosal integrity; inhibition of mucin synthesis, and stimulation pepsinogen secretion\(^{(19)}\).

2. Leukocyte recruitment and activating factors: These are soluble surface proteins produced by the organism that have chemotactic properties. They recruit neutrophils and monocytes to the lamina propria. They include H. pylori neutrophil-activating protein (nap), expressed by the napA gene\(^{(12)}\).

### iii) Virulence Factors of H. pylori

1. Vacuolating Cytotoxin (VacA): It is an exotoxin protein (encoded by the vacA gene) that causes vacuolation in eukaryotic cells\(^{(20)}\). VacA is a major virulence factors in the pathogenesis of H. pylori infection. VacA is secreted by a type V (autotransporter) secretion system. As a large 140-kDa polypeptide VacA precursor, it undergoes cleavage of an amino-terminal signal sequence and C-terminal proteolytic processing, resulting in an 88-kDa secreted passenger domain, a small secreted peptide, and a-barrel domain localized to the outer membrane\(^{(20)}\). The three most extensively studied regions of heterogeneity correspond to the signal “s” region, the intermediate “i” region, and the middle or “m” region of diversity within the 88-kDa passenger domain, within each region, sequences can be classified into one of two main types (s1 or s2, i1 or i2, and m1 or m2)\(^{(16)}\). VacA is found in all H. pylori strains, but 50% only express mature protein. In humans, nearly all isolated H. pylori strains are positive for VacA, it was documented that genotypes of vacA as the critical determinant of pathogenesis, rather than its presence or absence\(^{(22)}\). H. pylori can survive intracellularly as vacA assists in producing the vacuole which is the most important factor in disease pathogenesis\(^{(23)}\). VacA inserts itself into the epithelial cell membrane to form a hexameric anion-selective, voltage-dependent channel through which bicarbonate and organic anions can be released\(^{(24)}\). It also acts on the host mitochondrial membrane to induce apoptosis leading to release of cytochrome c from the intermembrane space\(^{(25)}\). VacA was related to H. pylori infection persistence since it inhibit the proliferation and immune response of T cells\(^{(14)}\).

2. Cytotoxin-Associated Antigen (CagA): Structure of CagA: CagA is a 120–145kDa protein encoded on the 40kb cag pathogenicity island (PAI)\(^{(26)}\). H. pylori strains can be divided into CagA positive or negative strains. Approximately 60% of H. pylori strains isolated in Western countries carry cag PAI, whereas almost all the East Asian isolates are cag PAI-positive\(^{(26)}\). The cag PAI also encodes for a type 4 secretion system which is used to “inject” CagA into a target cell upon H. pylori attachment. After translocation, CagA localizes to the inner surface of the cell membrane and undergoes tyrosine phosphorylation by Src family kinases\(^{(26)}\).

**Epidemiology of CagA:** The CagA prevalence among H. pylori in various regions differs greatly (e.g., 100% in east Asia and ≤50% in some western countries)\(^{(23)}\).

**Pathogenesis of CagA:** After bacterial attachment, CagA is trans-located into host cells by the type IV cag secretion system. Once entered the host cell, CagA is tyrosine phosphorylated [at the glutamate-proline-isoleucine-tyrosine-alanine (EPIYA) motifs] and induces cell morphological changes termed “the hummingbird phenotype,” a phenotype associated with cell elongation and cell scattering\(^{(27)}\). CagA is a very high immunogenic protein encoded by the cagA gene at one end of the cag pathogenicity island (PAI)\(^{(28)}\). The cag PAI is a 40-kb DNA insertion element that contains 27 to 31 genes flanked by 31-bp direct repeats\(^{(29)}\). At least 18 cag genes encode
components of Type IV secretion system (T4SS; i.e., a molecular syringe) that injects CagA and other proteins into mammalian cells where it triggers cytokine production (30). CagA protein can be further divided into the Western-type CagA and East Asian-type CagA, by the repeat sequence of EPIYA motifs at the N terminus of CagA. The affinity of the East Asian-type CagA to Src homology 2-containing protein-tyrosine phosphatase-2 (SHP-2) is significantly higher than that of the Western-type CagA. As a result, East Asian-type CagA induces more cytoskeleton changes, and is more likely to be associated with gastric cancer (31) (Figure II).

Four distinct EPIYA motifs (EPIYA-A, -B, -C, and -D) were found within the carboxy-terminal polymorphic region of CagA. They are different in the amino acid sequences surrounding the EPIYA motif (32).

Pathological features of CagA: The CagL protein is a specialized adhesion that binds and activates the integrin α5β1 receptor on gastric epithelial cells that trigger CagA delivery. CagA interacts with cytoplasmic SHP-2 (which has oncogenic activity) in the host cell (33). Antibodies to CagA can be used to detect CagA-producing H. pylori strains. The Cag PAI is associated with severe tissue inflammatory response and induces apoptosis via the mitochondrial pathway (34). Apoptosis of epithelial cells expose the epithelium to luminal acid and pepsin that is associated with increased risk of peptic ulcer disease (PUD) and gastric adenocarcinoma (35).

Effect of CagA over VacA and vice versa: CagA is able to down regulate the effects of VacA on host cell vacuolation, it also blocks VacA trafficking, preventing it from reaching its intracellular target and inducing vacuole formation. While VacA may down regulate CagA activity. Tyrosine-phosphorylated. Unphosphorylated CagA antagonized vacuolation by blocking VacA activity at the mitochondria. VacA also antagonizes the effects of CagA on cell scattering and elongation by inactivating epidermal growth factor receptor (EGFR) (36). In the absence of VacA and CagA, human gastric epithelial cells can be sensitized by H. pylori and become susceptible to TNF-related apoptosis-inducing ligand (TRAIL) mediating apoptosis (37).

3. Induced by Contact with Epithelium Gene (iceA): IceA is a gene whose transcription is up-regulated following adherence to gastric epithelial cells and encodes a CTAG-specific restriction endonuclease and encodes a CTAG-specific restriction endonuclease (38). It has two main allelic variants, iceA1 and iceA2. IceA1 genotype was linked with enhanced mucosal IL-8 expression and acute antral inflammation. Sensitivity analysis revealed that the iceA1 status was significantly associated with peptic ulcer (39).

4. Duodenal Ulcer (DU) Promoting Gene A (dupA): DupA is a risk marker for DU and a protective factor against GC (11). It is located in the plasticity region of H. pylori genome. It is associated with increased IL-8 production from the antral gastric mucosa in vivo as well as from gastric epithelial cells in vitro. Its presence is also involved in DNA uptake/DNA transfer and protein transfer (14).

5. Outer Membrane Protein (OMP): H. pylori genome contains more than 30 omp genes. They are divided into two subgroups: i) hop (Helicobacter OMPs) and ii) hor (hop-related groups) (40). Together with CagA, these proteins act to produce an intense inflammatory response (41). The Hop subgroup is encoded by 21 genes which include H. pylori adhesins: First, blood group antigen binding A (BabA) adhesin which binds to Lewis b (on gastric epithelial cells),
and cause severe disease\(^{(15)}\). Second, sialic acid binding adhesion mediates binding to sialyl-Lewis x, which is upregulated in inflammation\(^{(21)}\).

6. Heat shock proteins (Hsp): They are protein family detected in prokaryotes and eukaryotes. They are induced by environmental stresses as temperature, pH change, ischemia, and microbial infection. H. pylori produces two Hsps; Hsp10, and Hsp60\(^{(42)}\). Hsp were potential immunogens that induce IL-6, IL-8, TNF-α production from monocytes or gastric epithelial cells\(^{(43)}\).

**Figure 1:** Pathogenesis of Helicobacter pylori (H. pylori) infection. Several virulence factors, such as CagA and VacA, encoded by H. pylori genes, interact with gastric epithelial cells and the immune system, resulting in an inflammatory response, mucosal damage and, eventually, gastric cancerogenesis\(^{(44)}\).

**HELICOBACTER PYLORI AND GASTRIC CARCINOMA**

**Overview:** Infection of H. pylori is one of the thoroughly studied risk factors of GC\(^{(45)}\). Even though most people with H. pylori infection do not show any clinical indications, long term infection conceivably prompts irritation of gastric epithelium\(^{(46)}\).

**Potential risk factors for H pylori-related gastric carcinoma**

1. Events leading to GC following H. pylori infection: H. pylori infection of gastric epithelium leads to the development of intestinal-type adenocarcinoma with the primary event being the transition from normal mucosa to chronic superficial gastritis. Subsequently, atrophic gastritis ensues followed by intestinal metaplasia, leading to dysplasia and adenocarcinoma\(^{(47)}\).

Cag Pathogenicity Island and CagA: Cag Pathogenicity Island (cagPAI), was observed to be imperative in carcinogenesis since only H. pylori strains that contain cagPAI component increase the danger of atrophic gastritis and gastric tumor\(^{(48)}\). H. pylori CagA is translocated into host cells
following connection of the microbes to the cell. Inside the host cell, CagA is phosphorylated by Abl and Src kinases, on tyrosine residue at four distinct glutamate-proline-isoleucine-tyrosine-alanine (EPIYA) motifs present at the C-terminal region of the protein, leading to morphological changes in the cell(49). The number and phosphorylation status of these EPIYA motifs is a determinant and indicator of risk for GC(50).

**Peptidoglycan:** Along with CagA, H. pylori peptidoglycan can also be delivered into host cells and peptidoglycan binds with NodL(51), which triggers the NF-κB dependent pro-inflammatory pathway and IL-8 secretion(52). Other virulence factors present in H. pylori include VacA and outer membrane proteins, which are associated with ulceration as well as GC(53).

### 2. Inflammatory response to H. pylori infection

**COX-2/PGE2 pathway:** There are several components by which inflammation may advance cancer development. The induction of the cyclooxygenase-2/prostaglandin E2 (COX-2/PGE2) pathway and activation of NF-κB and Stat3 appear to be major pathways(54). Besides these, innate immune responses through the TLR/MyD88 adapter signaling also play a role in tumorigenesis(55). It has been demonstrated that all the gastric tumors demonstrate an induction of COX-2 expression(56) and H. pylori infection is known to prompt COX-2 expression(56). PGE2 signaling, through the EP4 receptor, is known to induce the expansion of CD133+ CD44+ cancer stem cells in intestinal tumors(57), which aggravates tumor growth. Infection of H. pylori induces inflammation through CagA infusion into host cells followed by the activation of SHP and TLRs, prompting unending dynamic gastritis and in the long run GC(57). However, the expression pattern of inflammation markers is not always comparable between gastritis and GC. Thus, IL-8 and IL-11 expression is predominantly induced in GC, whereas in gastritis mostly TNF-α expression is increased(57).

**IL-1β:** IL-1β is known to play a role in a variety of cellular activities such as inflammatory response and acid secretion by gastric epithelium(58). H. pylori infection leads to elevated secretion of IL-1β and reduction in acid secretion(59). Thus, infection of H. pylori promotes the expression of IL-1β, which leads to gastric carcinogenesis through its actions on both inflammatory and epithelial cells(60).

### 3. Oxidative stress induced by H. pylori

A primary factor that is important in the events that lead to the progression of the inflammation-to-carcinoma is oxidative DNA damage induced by H. pylori infection(61), which is probably due to infiltrating neutrophils, and also direct effects of H. pylori(62). Production of reactive oxygen species in the H pylori-infected gastric epithelium is linked to the presence of cagPAI and contribute to the oxidative stress response in gastric epithelial cells(63). It is well known that H. pylori infection causes elevated level of polyamines, in particular spermine and this is associated with an induction of spermine oxidase(64), that leads to the production of elevated levels of hydrogen peroxide, which is a powerful oxidizing agent and also contributes to the production of free radicals such as hydroxyl radical(65).

### 4. H. pylori and E-cadherin

**E-cadherin**, which is an adhesion molecule in epithelial tissues that is important in maintaining proper cellular architecture(66). It has been documented that there is a loss of E-cadherin function in GC, and in fact, promoter methylation of E-cadherin gene is induced by H. pylori infection, leading to reduction in E-cadherin expression(67). Following H. pylori infection, the
translocated CagA in the gastric epithelial cells ties with E-cadherin, bringing about the separation of the E-cadherin-β-catenin complex and aggregation of β-catenin in cytoplasm and core, where it transactivates β-catenin–dependent genes involved in carcinogenesis(7). Along with the downregulation of E-cadherin, a decreased expression or aberrant subcellular localization of p120, from membrane to the cytosol or nucleus, is commonly seen in GC(68).

5. Environmental factors and H pylori-mediated gastric carcinogenesis
Gastric adenocarcinoma is strongly influenced by dietary salt intake probably through elevating the production of inflammatory cytokines IL-1, IL-6 and TNF-α(69). It has been proposed that high salt increases the expression of CagA(70).

Figure 2: Tumor inflammatory microenvironment: Interplay of factors derived from H. pylori and tumor cells(71).

Summary
Gastric cancer is a major health burden worldwide. Development of gastric cancer involves host genetics, environmental factors, and H. pylori infection. There is increasing evidence from epidemiological studies of the association of H. pylori infection and specific virulence factors with gastric cancer. One major virulence factor in H. pylori is the cytotoxin-associated gene A (cagA), which encodes the cagA protein in the cag pathogenicity island (cag PAI).

Studying the association between the genotypes of H pylori virulence factors and gastric carcinoma would help in a subsequent reduced risk of gastric cancer.

References
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