H. pylori, a spiral-shaped bacterium that colonizes the human gastric mucosa, is estimated to inhabit at least half of the world’s human population. Since its first report in 1984 by Marshall and Warren, H. pylori has been recognized as the etiological agent of gastric diseases such as chronic atrophic gastritis and peptic ulcers. H. pylori infection is primarily acquired in childhood, transmission occurring through an oral–oral or fecal–oral mode primarily within families, and infection is lifelong in the majority of cases. Furthermore, the plasticity of the H. pylori genome generates a huge diversity of strains, with each strain showing differences in their genome sequence by more than 20%.[13] Thus, even a single strain may generate multiple variants and select those that adapt to an individual host environment during long-term colonization.[12] Despite the genomic diversity of H. pylori, epidemiological studies have revealed the importance of several genetic elements, such as cag pathogenicity islands (PAI), in the development of gastroduodenal disorders.

Gastric adenocarcinoma is the second most common cause of cancer-related deaths worldwide, with 876 000 estimated new cases and 405 000 estimated deaths in the year 2000.[3] Gastric adenocarcinoma is histopathologically subdivided into intestinal type and diffuse type. Intestinal-type gastric adenocarcinoma, which occurs in older people, is more common than diffuse type, which affects younger people with poor prognosis. A small subset of diffuse-type gastric adenocarcinoma is of familial origin, caused by mutations in the E-cadherin gene.[4] Recent epidemiological studies as well as infection studies using animal models have indicated that H. pylori plays a critical role in the development of both intestinal and diffuse types of gastric adenocarcinoma.[5–12] However, the molecular mechanisms by which H. pylori stimulates the development of gastric lesions leading to gastric carcinoma remain largely unknown.

H. pylori CagA, a bacterial intruder

H. pylori strains can be divided into two major subpopulations based on their ability to produce a 120–145-kDa immunodominant protein called cytotoxin-associated gene A (CagA) antigen.[13,14] The cagA gene that encodes CagA is localized at one end of the cag PAI, a 40-kb DNA segment that was most likely incorporated into the H. pylori genome by a process of horizontal transfer. Approximately 60% of H. pylori strains isolated in Western countries carry cag PAI, whereas almost all of the East Asian isolates are cag PAI-positive. The cag PAI DNA segment contains 31 putative genes (open reading frames), including cagA and those encoding components of a molecular ‘syringe’ termed the type IV secretion system,[15] through which macromolecules are delivered from the inside to the outside of the bacterium. Clinically, infection with the cagA-positive H. pylori strain has been associated with higher....

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grades of gastric mucosal inflammation as well as severe atrophic gastritis and has been suggested to play an important role in the development of gastric carcinoma. (16–23)

Upon attachment of cagA-positive H. pylori to the gastric epithelial cell, the CagA protein is injected directly into the cell via the cag PAI-encoded type IV secretion system (Fig. 1). (24–28) Translocated CagA then localizes to the inner surface of the plasma membrane, where it undergoes tyrosine phosphorylation by several members of the Src family kinases (SFK) such as c-Src, Fyn, Lyn and Yes. (29,30) Phosphorylation of CagA by SFK occurs in the absence of any stimuli, indicating that SFK are constitutively activated in gastric epithelial cells. In general, tyrosine phosphorylation plays a crucial role in transmitting intracellular signaling for growth, movement or differentiation in mammalian cells. Accordingly, the finding raised the intriguing possibility that, upon tyrosine phosphorylation, the bacterial protein disturbs signal transduction and thereby provokes cellular dysfunction that eventually leads to cell transformation.

**EPIYA motif, the site of CagA tyrosine phosphorylation**

The tyrosine phosphorylation site of CagA is characterized by the presence of a unique Glu-Pro-Ile-Tyr-Ala (EPIYA) motif, which is present in multiple numbers in the carboxy-terminal region of the protein (Fig. 2a). (31,32) From the sequences flanking these EPIYA motifs, four distinct EPIYA segments, EPIYA-A, -B -C and -D, each of which contains a single EPIYA motif, have been identified in the CagA protein. (32–34) The representative CagA protein of Western H. pylori isolates, such as those from Europe, North America and Australia, possesses a 32-amino acid EPIYA-A and a 40-amino acid EPIYA-B segment followed by a 34-amino acid EPIYA-C segment (‘A-B-C’-type CagA) (Fig. 2a). Intriguingly, the 34-amino acid EPIYA-C segment multiplies variably, mostly from one time to three times, among different Western CagA species as a result of homologous recombination or misaligned replication of a 102-bp cagA gene segment, which codes for the 34-amino acid EPIYA-C segment. The tyrosine residue that constitutes the EPIYA-C site is the major site of tyrosine phosphorylation in Western CagA by SFK in gastric epithelial cells, whereas those present within the EPIYA-A and EPIYA-B segments are only weakly phosphorylated in the cells. (31,32) Most CagA proteins of H. pylori isolated in East Asian countries such as Japan, Korea and China (East Asian CagA) also possess the EPIYA-A and EPIYA-B segments but not the repeatable EPIYA-C segment. Instead, they have a distinct EPIYA-containing sequence, termed the EPIYA-D segment, which is unique to East Asian CagA species (Fig. 2b). (32) East Asian CagA is therefore regarded as ‘A-B-D’-type CagA. The EPIYA-D site within the EPIYA-D segment represents the major tyrosine phosphorylation site of East Asian CagA. In addition to its role in tyrosine phosphorylation, the EPIYA motif acts as a membrane-targeting signal of CagA in gastric epithelial cells, although CagA–membrane interaction does not require EPIYA tyrosine phosphorylation. (34) Accordingly, the EPIYA

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Fig. 1. Physical and functional interaction between H. pylori CagA and SHP-2. CagA is translocated from H. pylori into H. pylori-attached gastric epithelial cells via the bacterial type IV secretion system. The translocated CagA protein localizes to the inner surface of the plasma membrane and undergoes tyrosine phosphorylation by Src family kinases. Upon tyrosine phosphorylation, CagA binds specifically to the SH2 domain-containing tyrosine phosphatase SHP-2 and activates the phosphatase activity. The CagA-activated SHP-2 potentiates Erk MAP kinase activity, which regulates both cell growth and cell morphology. SHP-2 also dephosphorylates a cellular substrate (shown as X in the figure), which is involved in induction of the hummingbird phenotype that is associated with elevated cell motility.
motif has a dual function in membrane association and tyrosine phosphorylation in CagA.

**SHP-2, a cellular target of tyrosine-phosphorylated CagA**

Upon tyrosine phosphorylation by SFK, CagA acquires the ability to bind specifically to SHP-2, a cytoplasmic protein tyrosine phosphatase having two tandem-repeated Src homology-2 (SH2) domains, termed N-SH2 and C-SH2, on the N-terminal half and a protein tyrosine phosphatase (PTP) domain on the C-terminal half (Fig. 1). The physical complex formed between CagA and SHP-2 is detectable not only in cells infected in vitro or transfected with CagA but also in gastric mucosa of patients infected with cagA-positive *H. pylori*. An SH2 domain functions as a protein module that specifically recognizes and interacts with a phosphotyrosyl peptide. Indeed, the CagA–SHP-2 interaction requires both of the functional N-SH2 and C-SH2 domains of SHP-2, indicating that two of the tyrosine-phosphorylated EPIYA sites, either in cis or trans, are actively involved in the stable complex formation between CagA and SHP-2. The crystal structure of SHP-2 indicates that the N-SH2 domain occludes the catalytic cleft of the PTP domain, blocking substrate access. This closed (inactive) structure keeps the basal phosphatase activity level of SHP-2 low. Binding of tyrosine-phosphorylated CagA to the SH2 domains induces a conformational change in SHP-2 that relieves inhibition of the PTP domain by the N-SH2 domain, resulting in the activation of SHP-2 phosphatase activity.

![Molecular anatomy of the EPIYA-containing region of CagA](image-url)

Fig. 2. Molecular anatomy of the EPIYA-containing region of CagA. (a) Subdivision of the EPIYA-containing region. Prevalent CagA proteins of Western *H. pylori* isolates contain three EPIYA motifs, which are designated EPIYA-A, EPIYA-B and EPIYA-C sites, based on the sequence surrounding each of the EPIYA motifs. Accordingly, the EPIYA-containing region of CagA can be subdivided into the EPIYA-A segment, the EPIYA-B segment and the EPIYA C segment. The border of each segment was determined by comparing sequences of the EPIYA-containing regions, which were made by extensive genetic recombination among various Western CagA isolates. (b) Comparison of the EPIYA-containing region between Western and East Asian CagA species. Both CagA species have the conserved EPIYA-A and EPIYA-B segments. Following the EPIYA-A and EPIYA-B segments, Western CagA possesses the EPIYA-C segment whereas East Asian CagA possesses the EPIYA-D segment, whose sequence is unique to East Asian CagA.
Pathophysiological consequence of the CagA–SHP-2 interaction

Numerous studies have indicated that SHP-2 participates in signal transduction downstream of growth factor and cytokine receptors to regulate cellular responses, including proliferation, morphogenesis and cell motility. Consistent with this finding, gastric epithelial cells expressing either infected CagA or transfected CagA elicit cell-morphological transformation, termed the hummingbird phenotype, which is characterized by elongated cell-shape with dramatic cytoskeletal rearrangements. Time-lapse video microscopic analysis has revealed that the hummingbird phenotype represents highly elevated motility of cells. Inhibition of CagA tyrosine phosphorylation or disruption of the CagA–SHP-2 complex inhibits induction of the hummingbird phenotype. Furthermore, constitutive or conditional knockdown of SHP-2 abolishes induction of hummingbird cells by CagA. Thus, CagA-deregulated SHP-2 plays a crucial role in induction of the hummingbird phenotype by CagA in gastric epithelial cells.

Physiologically, SHP-2 is known to activate Erk MAP kinase by both Ras-dependent and -independent mechanisms. Consistent with this, expression of CagA in gastric epithelial cells results in the sustained activation of Erk MAP kinase activity. As prolonged Erk activity has been suggested to play an important role in the G1 to S phase progression, CagA may predispose gastric epithelial cells to deregulated growth by inducing sustained Erk activation. Interestingly, Erk MAP kinase activity is also required for induction of the hummingbird phenotype by CagA, suggesting the role of sustained Erk activation in cell morphology and movement as well.

Differences in SHP-2 binding activity between East Asian and Western CagA proteins

East Asian CagA and Western CagA proteins possess distinctly structured tyrosine phosphorylation and SHP-2-binding sites, EPIYA-D and EPIYA-C, respectively. The sequence flanking EPIYA-D of East Asian CagA perfectly matches the consensus high-affinity binding sequence for the SH2 domains of SHP-2 (pY-[V/T/A/S]-X-[L/I/V]-X-[F/W]) (Fig. 3a). In contrast, the sequences flanking EPIYA-C of Western CagA differ from the consensus SHP-2 binding sequence by a single amino acid at the pY + 5 position. As a result, the EPIYA-D site in East Asian CagA exhibits stronger SHP-2 binding and greater morphogenetic activity than EPIYA-C of Western CagA. Among Western CagA species, the number of EPIYA-C sites is correlated directly with the levels of CagA tyrosine phosphorylation, SHP-2 binding activity and the morphogenetic activity of CagA. This finding suggests that Western CagA proteins possessing a greater number of EPIYA-C are biologically more active than those having a smaller number of EPIYA-C.

Inhibition of SFK by CagA

In addition to SHP-2, tyrosine phosphorylated CagA has been shown to bind to the SH2 domain of the C-terminal Src kinase (Csk). Through the interaction, CagA stimulates the kinase activity of Csk, which in turn phosphorylates SFK at the C-terminal inhibitory tyrosine residue. As a result, CagA inhibits SFK activity through Csk activation. Because SFK are kinases that phosphorylate CagA, this finding indicates the presence of a feedback mechanism that attenuates phosphorylation-dependent activities of CagA. Most notably, the CagA–Csk interaction may be important in limiting SHP-2 deregulation by CagA, which otherwise elicits acute and serious toxicity toward gastric epithelial cells. Thus, the negative feedback loop ensures a long-term equilibrium between cagA-positive H. pylori and the human host for decades without excess mucosal damage to the host.

In addition to Csk-mediated inhibition, CagA is capable of directly inhibiting SFK activity in a phosphorylation-dependent manner. CagA-mediated inhibition of SFK activity is associated with a decrease in the level of tyrosine-phosphorylated cortactin. Because cortactin plays a critical role in the actin cytoskeletal rearrangement, reduced tyrosine phosphorylation of cortactin by CagA may also play a role in induction of the hummingbird phenotype by CagA.

Phosphorylation-independent biological activities of CagA

Having shown that the membrane-bound and tyrosine-phosphorylated CagA specifically recruits SH2 domain-containing proteins such as SHP-2 and Csk, it can be concluded that the bacterial protein functionally mimics mammalian scaffolding adaptors such as Gab and insulin receptor substrate family proteins. Notably, however, there is no sequence similarity between CagA and any of the known mammalian proteins.

In addition to the functions of CagA as a phosphorylation-dependent scaffolding adaptor, recent studies have revealed phosphorylation-independent activities of CagA, which are also involved in cell growth and cell motility. CagA binds Grb2 and activates Ras in a manner independent of CagA tyrosine phosphorylation. The CagA–Grb2 interaction is also involved in elevated cell motility known as the scattering phenotype. Upon complex formation, CagA promotes proliferation of gastric epithelial cells through activation of the Ras-MAP kinase pathway. CagA also interacts with the c-Met hepatocyte growth factor (HGF) receptor. Although the complex formation requires infection of cells with H. pylori which causes tyrosine phosphorylation of c-Met, it is again independent of CagA tyrosine phosphorylation. The interaction between CagA and c-Met indicates that CagA potentiates HGF-dependent intracellular signaling that elicits cell morphological changes. Interaction of CagA with phospholipase C-gamma (PLCγ) has also been reported, although the functional consequence of the interaction remains to be elucidated.

Recent studies have revealed that translocated CagA associates with tight-junctional proteins such as ZO-1, causing an ectopic assembly of tight-junction components at sites of bacterial attachment and altering the function of the apical-junctional complex. Hence, long-term CagA delivery to polarized epithelia causes a disruption of the epithelial barrier function and dysplastic changes in epithelial cell morphology, alterations that have been shown to play a role in carcinogenesis.
Importantly, the CagA activity on apical junctions does not require CagA tyrosine phosphorylation. More recently, Franco et al. reported that β-catenin, a major component of adherens junctions, is deregulated in gastric epithelial cells expressing CagA. (48) Although the mechanism underlying β-catenin activation by CagA remains to be elucidated, results indicate that Wnt/β-catenin signaling, which plays a crucial role in colon carcinogenesis, is also involved in the development of gastric carcinoma.

Deregulation of transcription factors by CagA. CagA is capable of indirectly deregulating transcription factors through multiple distinct mechanisms. Hirata et al. showed that CagA activates serum responsive element-dependent transcription
in a manner independent of CagA phosphorylation.\(^{(49)}\) Brandt et al. recently showed that CagA is capable of activating NF-κB, which in turn induces interleukin-8 expression.\(^{(50)}\) As injection of the \textit{H. pylori}-derived proteoglycan via the type IV injection system of \textit{H. pylori} also stimulates NF-κB in gastric epithelial cells,\(^{(51)}\) these results indicate that \textit{H. pylori} activates NF-κB through multiple distinct mechanisms.

More recently, CagA was found to activate the nuclear factor of activated T cells (NFAT) in gastric epithelial cells.\(^{(52)}\) Expression of CagA in gastric epithelial cells activates the calcium-dependent serine/threonine phosphatase calcineurin and induces translocation of cytoplasmic NFAT to the nucleus, where it trans-activates NFAT-dependent genes. The CagA activity toward NFAT is again independent of CagA phosphorylation. Although the mechanism by which CagA activates the calcineurin-NFAT system needs further investigation, the reported CagA–PLC\(\gamma\) interaction might trigger Ca\(^{2+}\) mobilization and subsequent activation of calcineurin.\(^{(46)}\) Surprisingly, one of the NFAT-dependent genes activated by CagA in gastric epithelial cells is p21\(^{Cip1}\).\(^{(52)}\) Thus, whereas CagA activates a growth-promoting signal via SHP-2 deregulation, it simultaneously inhibits progression of the cell cycle through NFAT activation. As a result, CagA may cause proliferation, apoptosis or differentiation, depending on the cellular setting. Intriguingly, another \textit{H. pylori} virulence factor, vacuolating toxin VacA, counteracts the activity of CagA to activate NFAT.\(^{(52)}\) This finding indicates that VacA also plays a role in determining the fate of gastric epithelial cells expressing CagA (Fig. 4).

### Role of CagA in gastric carcinogenesis

Development of gastric adenocarcinoma is a multistep process that requires qualitative as well as quantitative alterations in the expression of oncogenes and tumor suppressor genes, lasting for several decades. During \textit{cagA}-positive \textit{H. pylori} infection, gastric epithelial cells are continuously exposed to the injection of CagA from the bacteria. The injected CagA binds and deregulates SHP-2 and other intracellular signaling molecules in both tyrosine phosphorylation-dependent and independent manners, generating deregulated signals for cell growth and cell movement. CagA also disrupts cell–cell junctions, destroying normal epithelial architecture.

Among the various CagA activities that disturb cellular functions, deregulation of SHP-2 by CagA is of potential importance in gastric carcinogenesis because mutations in \textit{PTPN11}, the gene encoding human SHP-2, have been identified in human malignancies.\(^{(53,54)}\) Most of the reported cases carry missense mutations in exons 3 and 8, which encode segments of the N-SH2 domain and the PTPase domain, respectively. Somatic SHP-2 mutations are found in 35% of cases of sporadic juvenile myelomonocytic leukemia, 5–10% of cases of childhood myelodysplastic syndrome, 7% of cases of B-cell acute lymphoblastic leukemia, 5% of cases of acute myelocytic leukemia and in some solid tumors such as neuroblastoma. Molecular modeling of SHP-2 indicates that such mutations weaken the autoinhibitory interaction and hence constitutively activate SHP-2 phosphatase activity. Accordingly, deregulation of SHP-2 by CagA functionally mimics the gain-of-function mutation of SHP-2 that is associated with human malignancies (Fig. 5). A potential role of SHP-2 in the development of gastric cancer has also been suggested by results of a recent study demonstrating that genetically engineered mice lacking the SHP-2 binding site on the interleukin-6 family coreceptor gp130 develop intestinal-type gastric adenocarcinoma with extremely high frequency.\(^{(55)}\)

![Fig. 4. A proposed model for functional interaction between CagA and VacA.](image-url)

(a) \textit{H. pylori}-injected CagA deregulates SHP-2 and other cellular target molecules that promote cell proliferation. Simultaneously, CagA activates NFAT and thereby induces NFAT-dependent genes such as p21\(^{Cip1}\). Elevated p21\(^{Cip1}\) then arrests gastric epithelial cells in G\(_1\) phase. Such G\(_1\)-arrested cells subsequently undergo senescence, apoptosis or intestinal trans-differentiation known as intestinal metaplasia. (b) When gastric epithelial cells simultaneously encounter VacA and CagA, VacA counteracts nuclear translocation of NFAT by CagA and thus abolishes induction of p21\(^{Cip1}\) in CagA-expressing cells. Accordingly, in the presence of adequate levels of VacA, CagA stimulates deregulated cell growth. (c) VacA inhibits production of T-cell growth factor, interleukin-2 (IL-2), by suppressing NFAT activity.
**CagA polymorphism and gastric cancer**

Whereas some human populations with high incidences of *H. pylori* infection, such as those in East Asian countries (Japan, Korea and China), have high incidences of gastric carcinoma, other highly infected populations, such as populations in central Africa, do not. This enigma might be explained at least in part by the structural polymorphism of CagA proteins among *H. pylori* strains circulating in different geographic areas. As noted previously, East Asian *cagA*-positive strains are at greater risk for gastric cancer than those infected with Western *cagA*-positive or *cagA*-negative strains (Fig. 6).

**cagA-positive** *H. pylori* infection induces progressive inflammatory changes in the gastric mucosa that leads to gastric cancer: superficial gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, carcinoma. Because CagA–SHP-2 complexes are detectable primarily in atrophic mucosa, the complex may be involved in the development of atrophic gastritis and the transition from atrophy to intestinal metaplasia. Possibly, CagA-triggered abnormal signals that deregulate cell growth, cell–cell contact and cell migration may enhance epithelial cell turnover as a result of increased cell proliferation and subsequent apoptosis. Such an elevated cell turnover obviously increases the risk of damaged cells acquiring precancerous genetic changes. In this regard, results of recent studies using a *Helicobacter*-infected mouse model have led to the surprising conclusion that gastric adenocarcinoma originates from circulating bone marrow-derived cells (BMDC), not from resident gastric cells. If this is also the case in humans, chronic mucosal inflammation caused by CagA-positive *H. pylori* may exhaust gastric stem cells and eventually lead to depletion of the resident stem cell pool, resulting in recruitment and settlement of BMDC into gastric mucosa. It has also been suggested that BMDC do not differentiate properly, resulting in progression to metaplasia, dysplasia and gastric cancer. This caveat obviously warrants further investigation.

**Fig. 5.** Involvement of SHP-2 in human malignancies. Point mutations in *PTPN11*, a human gene that encodes SHP-2, have been associated with childhood leukemias and some solid tumors. The mutations abolish the inhibitory interaction between the N-SH2 domain and the phosphatase domain of SHP-2 and therefore produce gain-of-function mutations. Upon complex formation with CagA, SHP-2 is fixed to its active state, mimicking the gain-of-function mutation of SHP-2.

**Fig. 6.** Classification of *H. pylori* based on the EPIYA-repeat polymorphism of CagA. *H. pylori* is divided into CagA-positive and CagA-negative strains. CagA-negative *H. pylori* is non-pathogenic or only weakly pathogenic and is not involved in gastric carcinogenesis. CagA-positive *H. pylori* is subdivided into those carrying East Asian CagA and those carrying Western CagA. Virulence of *H. pylori* carrying Western CagA is determined at least partly by the number of EPIYA-C. Those having CagA with greater number of EPIYA-C are more virulent and more closely associated with severe atrophic gastritis and gastric carcinoma than those having CagA with less EPIYA-C. *H. pylori* strains carrying East Asian CagA are biologically more active than most if not all Western-type *H. pylori* and individuals infected with this type of *H. pylori* are at the highest risk of developing gastric carcinoma.
morphogenetic activity of CagA. Thus, Western CagA proteins with a greater number of EPIYA-C sites are pathophysiologically more virulent and thus more carcinogenic. This notion has been supported by the results of recent work by Argent et al. demonstrating that five of six Western H. pylori strains harvested from gastric carcinoma patients possessed multiple EPIYA-C sites, whereas 18 of 19 Western H. pylori strains isolated from non-cancer patients possessed a single EPIYA-C site. (60)

Conclusions

Helicobacter pylori infection is declining as the standard of living rises. However, huge human populations have already been infected with cagA-positive H. pylori and are at a higher risk of developing gastric carcinoma in the short or medium term. Interaction of CagA with SHP-2, the first phosphatase that acts as a bona fide oncoprotein in human malignancy, is one of the key determinants for the development of gastric carcinoma associated with cagA-positive H. pylori infection.

References


CagA is divided into two major types: East Asian CagA and Western CagA. Further studies should make it possible to identify additional CagA polymorphisms that account for the differential degree of virulence. From the clinical standpoint, elucidation of cagA-positive H. pylori strains with the highest potential of developing gastric carcinoma will be extremely important. Recent studies have shown that eradication of H. pylori in humans appears to lower the risk of developing gastric carcinoma. (61) Thus, systemic eradication of oncogenic H. pylori from a high-risk population would dramatically reduce the worldwide incidence of gastric cancer. Furthermore, understanding molecular mechanisms underlying the H. pylori–gastric cell interaction is not only important in developing more effective therapies for gastric cancer but also crucial in understanding the genesis of inflammation-associated cancers.

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