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The *Helicobacter pylori cag* Pathogenicity Island

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Abstract

The *cag* pathogenicity island is a well-characterized virulence determinant. It is composed of 32 genes that encode a type IV bacterial secretion system and is linked with a more severe clinical outcome. The following chapters will explore the manipulation of bacterial factors in order to understand their role in gastric mucosal disease.

Keywords

Helicobacter pylori; *cag* pathogenicity island

1. The *Helicobacter pylori cag* Pathogenicity Island

H. pylori strains exhibit a high degree of genetic heterogeneity due to genomic rearrangements, point mutations, gene insertions, and/or deletions (1–4). Genetically unique variants of a single strain are present within the stomachs of each human host, and the genetic composition of these populations can evolve over time (5). The identification of bacterial factors clearly associated with disease outcomes has been hindered because of this high level of genetic diversity; however, specific loci have been identified that augment the risk for carcinogenesis. The *cag* pathogenicity island (*cag* PAI) is a 40 kb DNA insertion element, containing approximately 32 genes that encode a bacterial type IV secretion system (4, 6–8). The *cag* PAI is a well-characterized *H. pylori* virulence determinant that is present in approximately 60–70% of Western *H. pylori* strains and virtually 100% of East-Asian *H. pylori* strains (4, 6–8). Although all *H. pylori* strains induce gastritis, strains that harbor the *cag* PAI (*cag*$^+$) augment the risk for severe gastritis, atrophy, dysplasia, and gastric adenocarcinoma compared to strains that lack the *cag* island (*cag*$^-$) (9–20). *H. pylori cag*$^-$ strains are found predominantly in the mucus gel layer while *cag*$^+$ strains are found adjacent to or adherent to gastric epithelial cells, indicating that *cag* genotype influences the topography of colonization within the stomach (21).

The *cag* type IV secretion system allows for the delivery of bacterial effector molecules into host gastric epithelial cells. One of these proteins, CagE, is a structural component of the functional type IV secretion system and inactivation of this gene product abrogates delivery of *H. pylori* proteins into host cells. Another component of the secretion system, CagL, functions as a specialized bacterial adhesin that binds to and activates $\alpha_5\beta_1$ integrin receptors, triggering the delivery of bacterial effector molecules into the cytoplasm of host cells (22). CagL bridges the type IV secretion system to $\alpha_5\beta_1$ integrins on target cells and activates host focal adhesion kinase (FAK) and Src (Fig. 1). In addition to $\alpha_5\beta_1$ integrin, CagL can also bind integrin and fibronectin, although the downstream consequences of binding to these receptors remains undefined. Recently, additional Cag proteins (CagA, CagI, CagY) have been shown to bind β_1 integrin and induce conformational changes of integrin heterodimers, permitting translocation of bacterial effectors (23).

2. CagA

The terminal gene product of the *cag* island, CagA, is translocated into host cells by the *cag* type IV secretion system following bacterial attachment (24). Transgenic mice that overexpress CagA develop gastric epithelial cell hyperproliferation and gastric adenocarcinoma (25), implicating this molecule as a bacterial oncoprotein. CagA is a 120–140 kD protein that contains tyrosine phosphorylation motifs (glutamate-proline-isoleucine-tyrosine-alanine, EPIYA) within the carboxyl-terminal variable region of the protein (26). To date, four distinct EPIYA motifs have been identified within the carboxyl-terminal polymorphic region of CagA. These motifs are designated EPIYA -A, -B, -C, or -D and are distinguished by the amino acid sequences flanking the EPIYA motif (27–29). Most variants of CagA throughout the world contain EPIYA-A and -B motifs, while EPIYA-C motifs are predominantly found in strains from Western countries (Europe, North America, and Australia). The number of EPIYA-C sites can vary; however, most CagA proteins contain a single EPIYA-C site (A-B-C-type). The EPIYA-A and -B motifs are phosphorylated to a lesser extent than EPIYA-C motifs and in Western strains an increased number of CagA EPIYA-C motifs correlates with increased gastric cancer risk (30, 31). EPIYA-D motifs are found almost exclusively in East-Asian *H. pylori* strains (Japan, Korea, and China) and are phosphorylated to a greater extent than all other EPIYA motifs (27). *H. pylori* strains containing EPIYA-D motifs induce significantly higher levels of IL-8 release from gastric epithelial cells compared to strains harboring Western A-B-C-type CagA (27, 32). Thus, the majority of *cag*$^{+}$ Western strains express A-B-C-type CagA, and the number of EPIYA-C regions may vary between 1 and 3 repeated copies among different strains, while East-Asian strains typically express A-B-D-type CagA (27).

3. CagA Phosphorylation-Dependent Perturbation of Host Cell Signaling

Following its injection into epithelial cells, CagA undergoes tyrosine phosphorylation at EPIYA motifs by members of the Abl and Src family of kinases (24, 33–37) (Fig. 1). Intracellular, phosphorylated-CagA, in turn, activates a eukaryotic phosphatase (SHP-2) and extracellular signal-regulated kinase 1 and 2 (Erk1/2), leading to cell scattering, robust actin reorganization, and other morphologic changes reminiscent of unrestrained stimulation by growth factors (24, 26, 33–41). Specifically, CagA transfection studies have demonstrated that phosphorylated-CagA-SHP-2 interactions contribute to cytoskeletal rearrangements and cell elongation by activation of the Erk-signaling pathway (38). East-Asian A-B-D-type CagA exhibits a higher binding affinity for SHP-2 than Western A-B-C-type CagA and, therefore, induces a more robust morphologic response by gastric epithelial cells (39).

H. pylori CagA proteins tightly and specifically regulate the activity of Src and Abl family kinases in a time-dependent manner. Src is activated during the initial stages of infection and is then rapidly inactivated, while Abl is continuously activated by *H. pylori* with enhanced activities at later time points, supporting a model of successive phosphorylation of CagA by Src and Abl family kinases (42). Phosphorylated-CagA can also inhibit Src via recruitment of C-terminal Src kinase (Csk), a negative-regulator of Src that acts rapidly to initiate a negative feedback loop to downregulate Src signaling (41, 43) (Fig. 1). As Src is the primary kinase activated by CagA, inhibition of Src by phosphorylated-CagA generates a negative feedback loop that carefully regulates the amount of intracellular, phosphorylated-CagA. The catalytic activity of Src is inhibited by phosphorylated-CagA, leading to tyrosine dephosphorylation of the actin-binding proteins cortactin, ezrin, and vinculin, which ultimately results in cellular rearrangements and elongation (43–45).

In AGS human gastric epithelial cells, translocation and subsequent phosphorylation of CagA results in cell elongation and scattering, known as the “hummingbird” phenotype (35,

46). In this cell line, interactions between phosphorylated-CagA and SHP-2 increase the duration of Erk activation in a Ras- and PI3K-independent manner, resulting in cell elongation (38). The interaction between phosphorylated-CagA and SHP-2 also results in dephosphorylation and inactivation of FAK, which again leads to morphologic aberrations (47) (Fig. 1).

4. CagA Phosphorylation-Independent Perturbation of Host Cell Signaling

Non-phosphorylated CagA also exerts numerous effects within gastric epithelial cells that contribute to pathogenesis. CagA translocation, but not phosphorylation, leads to disruption of apical–junctional complexes (Fig. 1). Non-phosphorylated CagA associates with the epithelial tight-junction scaffolding protein zona occludens 1 (ZO-1) and the transmembrane protein junctional adhesion molecule A (JAM-A), leading to nascent but incomplete assembly of tight junctions (TJ) at ectopic sites of bacterial attachment (48). In addition, non-phosphorylated CagA disrupts adherens junctions (AJ) leading to aberrant activation of β-catenin and an overall loss of barrier function and cellular polarity (48–53), alterations that play an important role in carcinogenesis. Non-phosphorylated CagA interacts with the cell adhesion protein E-cadherin, the hepatocyte growth factor receptor c-Met, the phospholipase PLC-γ, and the adaptor protein Grb2 (51, 52, 54, 55), which leads to proinflammatory and mitogenic responses, disruption of cellular junctions, and loss of cellular polarity. Recently, non-phosphorylated CagA was shown to directly bind PAR1b/MARK2, a central regulator of cell polarity, and inhibit its kinase activity. This interaction induced the dysregulation of mitotic spindle formation, promoting a loss of cellular polarity (52, 56, 57). These events were dependent on conserved 16 amino acid repeat motifs embedded within the 3 terminus of CagA and which are known as CagA multimerization (CM) sites (58), the conserved repeat responsible for phosphorylation-independent activity (CRPIA) (59), or the PAR1b/MARK2 kinase inhibitor (MKI) (60) motifs. These motifs, which vary in number among *H. pylori* strains, bind PAR1b/MARK2 and mediate homodimerization of CagA, conferring heightened SHP-2 binding and activation. A recent co-crystallography analysis of the CagA-PAR1b/MARK2 complex demonstrated that the PAR1b/MARK2-binding site resides in the initial 14 amino acids of the CagA CM motif, and binding leads to inhibition of PAR1b/MARK2 kinase activity (60).

5. Peptidoglycan

Another consequence of *H. pylori cag* pathogenicity island-mediated host cell contact is the production of pro-inflammatory cytokines. In certain *H. pylori* strains, CagA can induce IL-8 expression via NF-κB activation (61–63); however, the ability of CagA to induce IL-8 expression is not universal across all *cag* PAI-bearing strains (64–67). In addition to CagA, *H. pylori* peptidoglycan (PGN) is delivered into host cells via the *cag* type IV secretion system and outer membrane vesicles (OMV) (68), where they are sensed by the nucleotide-binding oligomerization domain 1 (NOD1), an intracytoplasmic pathogen pattern-recognition molecule (69, 70) (Fig. 2). NOD1 activation by *H. pylori* peptidoglycan stimulates the production of proinflammatory cytokines MIP-2, β-defensin, and IL-8 through induction of host cells signaling molecules, nuclear factor κB (NF-κB), p38, and Erk (70, 71). Furthermore, NOD1 activation by *H. pylori* peptidoglycan also regulates the production of type I interferon (IFN), which likely affects Th1 cell differentiation (72). NOD1-deficient mice develop an attenuated mucosal cytokine response following infection with *H. pylori cag*$^+$ strains (73), implicating peptidoglycan-NOD1 signaling as an important mediator of *H. pylori* pathogenesis.

Delivery of peptidoglycan components into host cells induces additional epithelial responses with carcinogenic potential, such as activation of PI3K and cell migration (Fig. 2). The *H.*

pylori gene *slt* encodes a soluble lytic transglycosylase that is required for peptidoglycan turnover and release (70), thereby regulating the amount of peptidoglycan translocated into host cells. Inactivation of *slt* has now been shown to inhibit *H. pylori*-induced PI3K signaling and cell migration (74). The protein encoded by the *H. pylori* gene *HP0310* deacetylates *N*-acetylglucosamine peptidoglycan residues and is required for peptidoglycan synthesis (75). Loss of *HP0310,* leading to decreased peptidoglycan production, reciprocally augments delivery of the other major *cag* secretion system substrate, CagA, into host cells, suggesting that functional interactions occur between *H. pylori* translocated effector molecules (76). Further, *H. pylori* peptidoglycan deacetylation by HP0310 is an important mechanism for mitigating host immune detection, which facilitates bacterial persistence and colonization (77). In total, these findings indicate that contact between cag^+ strains and host cells activates multiple signaling pathways that regulate oncogenic cellular responses, which may heighten the risk for transformation, particularly over prolonged periods of *H. pylori* infection.

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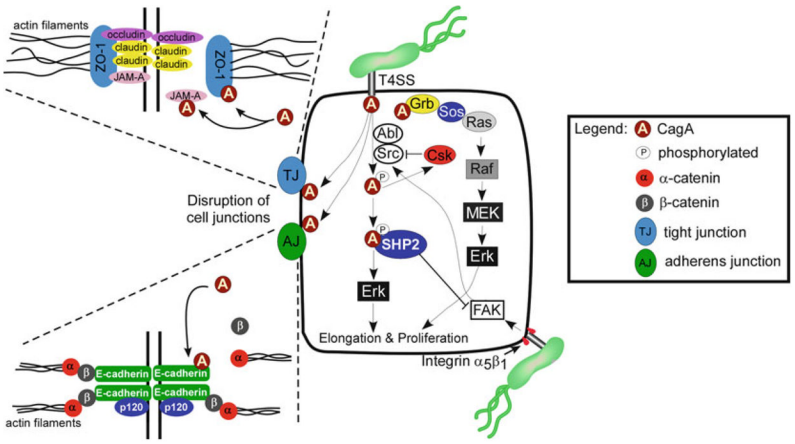

Fig. 1.

Molecular signaling alterations induced by intracellular delivery of CagA. Translocation of CagA by the *H. pylori cag* type IV secretion system leads to activation of host signaling pathways that promote epithelial responses with carcinogenic potential. CagA is phosphorylated by Src and Abl kinases, which is followed by a decrease in levels of phosphorylated-CagA via the inhibitory kinase c-src kinase (Csk). Phosphorylated CagA activates SHP2 and Erk leading to morphological changes, such as cellular elongation. Additionally, the interaction between phosphorylated-CagA and SHP2 results in inactivation of focal adhesion kinase (FAK), which can activate Src. Unphosphorylated CagA also leads to changes in epithelial cell motility and proliferation through binding Grb/Sos/Ras and activation of the Raf/MEK/Erk pathway. Unmodified CagA can also associate with the tight junction proteins ZO-1 and JAM-A as well as the adherens junction protein E-cadherin, leading to dysregulated junctional complexes.

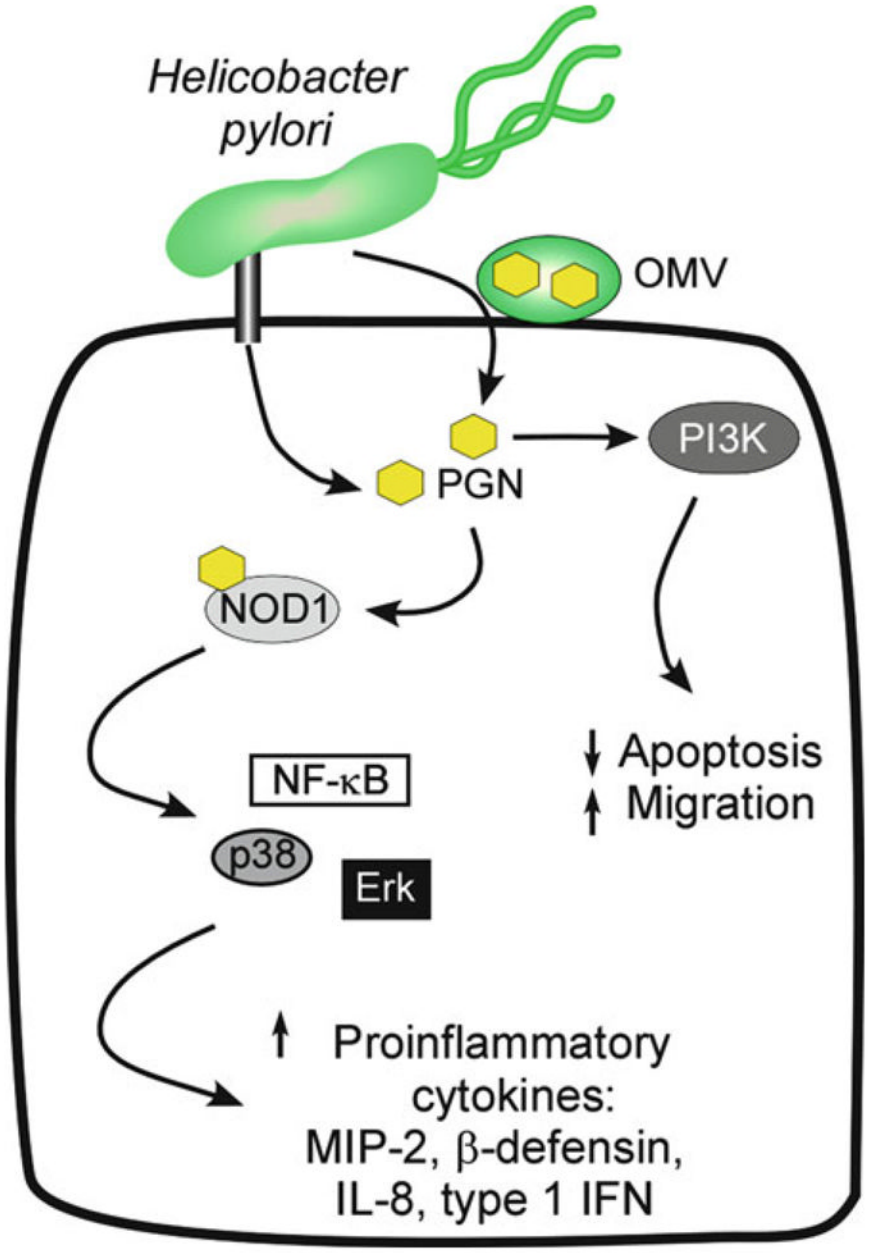

Fig. 2.

Molecular signaling alterations induced by intracellular delivery of peptidoglycan. In addition to CagA, the *H. pylori cag* type IV secretion system can deliver peptidoglycan (PGN) into host cells. Another mechanism of PGN delivery is via outer membrane vesicles (OMV). Delivery of PGN results in activation of the intracellular receptor nucleotide oligomerization domain 1 (NOD1) and triggers multiple signaling pathways that culminate in NF-κB activation and subsequent production of inflammatory and immune effectors, such as IL-8 and Type 1 IFN. Further, PGN can also activate PI3K, leading to decreased levels of apoptosis and increased cell migration.