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Review Article

The VacA and The CagA of *Helicobacter Pylori*: Two Multitasking Proteins of a Multitasking Bacterium

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Abstract

Helicobacter pylori infects 50% of the population and 10-20% of the infected individuals develop various gastroduodenal illness, which include gastritis, duodenal ulcer, gastric ulcer, distal gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma. The bacterium was classified as type I carcinogen by WHO in 1994. Most of the pathogenic potentials of *H. pylori* have been attributed to two virulence factors, namely the vacuolating cytotoxin (*vacA*) and the cytotoxin-associated gene A (*cagA*). For some geographical regions, however, carrying the virulence specific genes and expressing them may not correlate with the *H. pylori* associated clinical manifestations. Moreover, most of the *H. pylori* infections are benign and eradication of the bacterium may increase the probability of having other diseases, like esophageal cancer. The reason for these extreme variations in clinical outcomes in relation to *H. pylori* infection is unknown, which underscores the need for studying the mechanism of developing these diseases by multidisciplinary approach.

Introduction

There are some qualities—some incorporate things,
That have a double life, which thus is made
A type of that twin entity which springs
From matter and light, evinced in solid and shade.
-Edgar Allen Poe

Since the first culture of *Helicobacter pylori* from human gastric biopsy in 1983, a robust association has been shown with this bacterium and the causation of peptic ulcer, gastritis and gastric adenocarcinoma[1,2]. Instead of ablation of the stomach, the treatment of peptic ulcer diseases now consists of a short course of antibiotics coupled with proton pump inhibitors. *H. pylori* colonization is associated with gastric cancers and eradication of the bacterium has been shown to cure mucosa-associated lymphoid tissue lymphoma[3]. Later, *H. pylori* infection was also linked with extragastric diseases like cardiovascular disorder, neurodegenerative disorders and rheumatoid arthritis[3]. Therefore, treatment and eradication of *H. pylori* from all infected individuals have been suggested[4]. But on the other hand, a considerably good body of literature suggests that the colonization of *H. pylori* could actually be protective against gastro-esophageal reflux disease, Barrett's esophagus, esophageal adenocarcinoma as well as asthma[5,6]. Also, we do not understand why ~80% of the *H. pylori* infected individuals remain asymptomatic throughout their lives. Thus, the true personality of this spiral gastric colonizer still appears to be dual or indefinite—lying somewhere between 'Dr. Jekyll and Mr. Hyde'. Quite reasonably, most of the *H. pylori* research articles have been directed to reveal the 'Mr. Hyde' side of this bacterium. However, in spite of tremendous research leading *H. pylori* as the most cited bacterium for the past three decades, the exact mechanisms of developing various clinical manifestations sill remained unresolved. This review aims to discuss some of the exciting, but paradoxical facts that are present in literature on two well known virulence factors of *H. pylori*, the vacuolating cytotoxin (*vacA*) and the cytotoxin-associated gene A (*cagA*), in relation to heterogeneous clinical outcomes.

The vacuolating cytotoxin (VacA)

Discovery.

The first *H. pylori* toxin was discovered even before the bacterium was named *H. pylori* (that time the bacterium was named as *Campylobacter pylori*). In 1988, Leunk and colleagues found that the cell free broth-culture filtrates of 55% of the strains produced cytopathic effects in 7 of 9 cell lines that were tested for the assay[7]. HeLa and intestine 407 cells were highly responsive; HEp-2, WiDR, 5637, Vero and KATOIII cells were moderately responsive; Y-1 and CHO-K1 cells were unresponsive. The cellular response consisted of large membrane bound intracellular vacuoles as revealed by phase-contrast microscopy and transmission electron microscopy. The cell free substance

that possessed the vacuolating activity was sensitive to protease and was precipitable with ammonium-sulphate, indicating that it was a secreted protein toxin. Several years later, in 1992, the vacuolating cytotoxin (VacA) of *H. pylori* was purified from the cell free broth culture supernatant[8].

The *vacA* gene alleles and clinical outcomes.

All *H. pylori* strains carry a copy of *vacA*, but not all strains secrete the toxin or produce vacuolation in cells[9]. The *vacA* gene can be divided into four major parts: (a) a region near 5' end encoding the signal sequence (s); (b) the intermediate region (i); (c) the mid region (m); and (d) the 3' end encoding a type-V auto-transporter[10]. Three of these regions (s, i and m) show allelic polymorphism among *H. pylori* strains. The s region of *vacA* encodes for either s1 or s2 signal sequence of VacA precursor protein[11]. Only the toxin encoded by s1 allele carrying *vacA* gene produces vacuoles, while the toxin that is encoded by the s2 allele, due to an additional hydrophilic stretch of 12 amino acids, lacks this activity[12,13]. Consistently, the s1 allele, but not the s2 allele, of *vacA* has been shown to be associated with diseases like ulcer and cancer in the West[14]. Likewise, the mid region of the *vacA* gene can be divided into m1 and m2 (11). Any allelic combinations of signal sequences and mid-regions are possible (like s1m1 or s1m2 or s2m1 or s2m2) but s2m1 combinations are rare[11]. The secreted toxin produced from the *vacA*m1 and the *vacA*m2 show differences in cell tropism due to a 148-residue region[15,16]. The VacA encoded by m1 alleles can produce vacuoles in HeLa as well as RK13 cells, while the m2 allele express a protein that can generate vacuole in RK-13 cells, but not in HeLa cells since it fails to bind to HeLa cells[17]. The *vacA*s1m1 allele is more prevalent in most geographic regions (except in South East Asia) and is reported to be associated with ulcer and cancer as compared to *vacA*s1m2[18]. Between the signal sequence and the mid-region, the *vacA* contains the intermediate region, which may have three allelic variations— i1, i2 and i3. Recent analyses suggest that the i1 is associated with more vacuolating activity and severe clinical outcome[19-21].

The VacA protein.

The 149 kDa VacA precursor protein has a ~33-residue signal peptide, a 88 kDa toxin and a 50 kDa type V autotransporter protein[22]. Upon sequential proteolysis at both the N and the C terminal regions, only the 88 kDa middle portion is secreted as toxin with the help of the type V secretion system [10]. Cryo-electron microscopic (cryo-EM) analyses revealed that the secreted VacA may form a water soluble hexameric flower shaped molecule (also suggested as dodecameric aggregate). However, only the VacA monomer, which is seen in acidic pH, binds to the host cell membrane and gets internalized[23,24]. The secreted toxin is significantly stable at low pH (up to 1.5) and at high temperature (56ºC for 30 min)[7,25]. The 88 kDa VacA has two distinct domains, p33 (33 kDa; residues 1 to 311) and p55 (55 kDa; residues 312 to 821)[26]. These two domains are sometimes proteolytically cleaved and noncovalently associated, but the cleavage is not required for activity[24]. The p33 and the p55 domains can interact with each other[27]. The solved 2.4 Å crystal structure of the VacA p55 domain revealed features of autotransporter passenger domains, like predominance in right-handed parallel beta-helix[26]. Docking the crystal structure of p55 into a cryo-EM map of the VacA oligomer suggests that the p33 domains forms a ring shaped structure in the oligomer and interacts with the N-terminal portion of several p55 domains within the VacA oligomer[26,28].

Biological functions of the VacA.

The VacA is a multifunctional protein, which interferes with several cellular processes including cell death and immune response (Figure 1). Earlier studies showed that the VacA causes the appearance of acidic vacuoles, which are linked to cytoskeletal elements[29,30]. The vacuoles are rich in rab7, a marker for the late endosomes or early lysosomes[30,31]. The VacA is hypothesized to form anion selective channels in these vesicles and hence is classified as 'pore-forming toxin'[10,31]. Finally, the anion selective channels facilitate chloride ion transport, which results in diffusion of membrane-permeable weak bases, swelling and vacuolation[10]. Several regions of the VacA (like a hydrophobic region near the N-terminal domain, residues 6–27 in p33; a region within the p55, residues 394-422) has been shown to be important, but neither the p33 nor the p55 alone can form vacuoles[10,24]. Presumably, the entire p33 and the N terminal region of the p55 (residues 1-422 or 1-672 according to another report) are required for comprehensive vacuolating activity[24,32].

The VacA is also shown to be localized in mitochondria and due to its pore-forming activity is capable of releasing cytochrome c, a key event that precedes the apoptosis via intrinsic pathway[10]. Consistently, change in mitochondrial electrochemical membrane potential, the induction of proapoptotic proteins like Bax and Bak as well as cleavage of caspase 3 were confirmed[33-35]. Importantly, this cell death is independent of cell vacuolation[34]. On the other hand, the activation of caspase 8 and caspase 9 mediated apoptosis and caspase-independent programmed necrosis promoting action of VacA were also reported[36,37]. Another study suggested that the low-density lipoprotein receptor-related protein-1 (LRP1) can act as VacA receptor and VacA internalization through this receptor, but not through other receptors like RPTPα, RPTPβ, and fibronectin, facilitates autophagy[38]. The VacA is able to induce p38 mitogen-activated protein kinase (MAPK)/ activating transcription factor 2 (ATF-2) mediated signal transduction pathway, which is also independent of cell vacuolation[39]. The activation of β-catenin signaling by the VacA depends on phosphorylation of Akt at Ser473 in a PI3K dependent pathway[40]. Finally, the *H. pylori* VacA has strong immunomodulatory actions as it is capable of inhibiting stimulation-induced proliferation of CD4(+) T cells, CD8(+) T cells, and B cells[41,42].

The cytotoxin-associated gene A (*cagA*).

Discovery.

The CagA protein was discovered because of its strong immunogenicity. Human serum derived from patients, who were infected with toxigenic (VacA expressing) strains of *H. pylori* reacted strongly with a bacterial protein as discerned by Western blot analyses and ELISA[43,44]. The protein showed slower migration (120-128kDa) than VacA (88-90kDa) in SDS-PAGE and was considerably more immunoreactive than VacA[44]. It was predicted that the expression of this protein could be strongly associated with the expression of VacA and could be associated with severe clinical outcome[44]. In 1993, the gene that encodes the 120-128kDa immunodominant protein was cloned and expressed by two different groups and since its expression seemed

associated with the expression of the vacuolating cytotoxin, the gene was named cytotoxin-associated gene A (*cagA*)[45,46].

The *cagA* gene polymorphisms and clinical outcomes.

Analyses of the flanking region of the *cagA* revealed that the gene is part of a horizontally acquired pathogenicity island (PAI), which is present in ~50–70% of the Western and ~90% of the Asian *H. pylori* strains[47]. The presence of *cag*PAI is strongly associated with the presence of *vacA*s1m1 as well as more severe clinical outcome. The *cag*PAI shows characteristics that are similar to other bacterial PAIs, like different G+C content (~35%) from the rest of the genome (~39%), direct repeats and insertion sequences (eg. IS605)[47]. The ~40kb *cag*-PAI contains genes that encodes a type-IV secretion system and genes that are responsible for inducing IL-8 secretion[47].

The *cagA* is present at the right end of the *cag*PAI. The *cagA* has a conserved 5' end region but a highly polymorphic 3' end region. Phylogenetic analyses using 5' end conserved nucleotide sequence shows that the *cagA* of Western *H. pylori* strains and the *cagA* of East Asian *H. pylori* strains are different[48]. However, the differences in sequences are more significant at the 3' ends of the *cagA* genes of Western and East Asian strains[49,50]. The *cagA* 3' end encodes a stretch of five amino acids motif, EPIYA, and the tyrosine (Y) residue within this motif can become phosphorylated[49]. The number of the EPIYA motifs (usually 3-5 for most strains, but 1 and 2 EPIYA motifs were also reported) and the amino acid sequences among the EPIYA motifs vary between the Western and East Asian *H. pylori* strains[18,49,51]. The presence of East Asian specific sequence (ESS) in CagA primary structure is hypothesized to be the reason for occurrence of more gastric cancer cases in Japan due to stronger intracellular response than the Western specific (WSS) CagA sequence[49,50]. Between any two EPIYA motifs presence of several discrete motifs (possibly due to recombination events at the 3' end of the *cagA*) were also reported[50-53]. Based on PCR, the *cagA* 3' end region that encodes the EPIYA motif and adjacent repeat units can be typed and different nomenclatures were proposed[50-52].

The CagA protein

Purified CagA, upon partial digestion with V8 protease of *Staphylococcus aureus* results in generation of two fragments—a 100-kD N-terminal region and a ~35-kD C terminal region[54]. It is due to the variation in sizes of the C-terminal region, the molecular weight of the CagA varies (120-140kDa). For the N-terminal region of CagA, two crystal structures, one at 3.6Å (for residues 1-884) and another at 3.19Å (for residues 1-876), have been solved[54-55]. According to one report, the N terminal region comprised of four domains or conserved surface-exposed patches (CSP4) that are arranged in a "crescent moon" shape[55]. Whereas, the other report mentioned that the overall N terminal region contains three domains (domain I, residues 24-221; domain II, residues 303-644 with a subdomain composed of residues 370-446; domain III, residues 645-824) and these domains form a square plate-like shape[54]. Residues 782-820 within the N-terminal region and residues 998-1038 within the C-terminal region mediate the intramolecular interactions of CagA[54]. The EPIYA motifs containing C-terminal region of the CagA is intrinsically disordered and carries features (like PPII conformation) for scaffolding proteins[54,56]. A 16-residue motif (FPLXRXXXVXDLSKVG) within this region is involved in CagA multimerization within the host cell but this multimerization may occurs through the interaction with cellular proteins[56,57].

Biological functions of the CagA.

The *H. pylori* CagA is a master regulator that can hijack host signaling through protein-protein interactions by phosphorylation dependent or phosphorylation independent manner (Figure 1). The CagA is injected to the host cell by the *cag*PAI encoded type IV secretion system with a process that requires host transmembrane protein integrin. Integrins can interact with several proteins that are encoded by *cag*PAI including the N terminal region of the CagA[55,58]. The N-terminal region can interact with several other intracellular partners, such as ASPP2, RUNX3, TAK1, and TRAF6 and modulates cellular pathways.

However, the protein-protein interactions that are mediated through the CagA C terminal region are most well studied. The CagA, after being localized to the inner surface of the plasma membrane, undergoes tyrosine phosphorylation within the EPIYA motifs by the Src family protein tyrosine kinase[59]. Phosphorylated CagA interacts with the SH2-domain-containing protein tyrosine phosphatase (SHP2) leading to deregulation of this *bona fide* oncoprotein[60]. This event is necessary and sufficient for hummingbird phenotype and possibly for developing cancer, particularly in East Asia[50,60,61]. Transgenic expression of the CagA induces gastric and hematopoietic neoplasms in mouse, but phospho-resistant CagA failed to develop these tumors[62]. This phosphorylation dependant interaction occurs through EPIYA-C and EPIYA-D segments, while the EPIYA-A and EPIYA-B segments can be involved in interacting with the C-terminal Src kinase (Csk)[49]. CagA-Csk interaction inhibits the Src dependent phosphorylation of CagA by a negative feedback loop[63]. The CagA EPIYA motifs can also regulate cellular pathways by interacting with SHP1, Grb2, Grb7, PI-3 kinase, and Ras-GAP1 in a tyrosine phosphorylation-dependent manner[49,64]. Among these, Grb2 can also interact to the CagA in phosphorylation-independent manner and block intrinsic apoptosis by a mechanism that involves host elements like CRK, MEK, SRE, and SRF[65,66]. The CagA multimerization, which is independent of phosphorylation, is a prerequisite for the interaction of SHP2[57]. The CagA disrupts TJs and induces invasiveness of AGS gastric carcinoma cells via Cdx2-dependent targeting of Claudin-2 and associates with the epithelial tight-junction scaffolding protein ZO-1, which leads to disruption of the epithelial apical-junctional complex[67,68]. Moreover, a fraction of the internalized CagA goes to mitochondria and induces the production of reactive oxygen species, which leads to a series of events including mitochondrial DNA damage, oxidative stress to the gastric mucosa, nuclear DNA damage and all these events ultimately facilitate carcinogenesis[69].

Perspective and concluding remarks

The VacA and the CagA, both of these virulence factors of *H. pylori*, are champions in multitasking. These multi-domain proteins interact with many intracellular proteins and regulate signaling pathways that participate in crucial decision making, like cell death or cell survival and immune response or immune suppression (Figure 1). Interestingly, the effects of these two toxins on several cellular pathways are apparently counteracting. Antagonistic effect between the CagA the and VacA have

been shown in cellular processes like NFAT pathway, apoptotic activity and VacA traffic to intracellular compartments[70]. Why then the expression of the CagA is highly correlated with the expression of VacA? Numerous studies have highlighted the association of *vacA*s1m1 and *cagA* with diseases. This association, however, seem to vary with geographic regions[18]. For example, strains carrying the *vacA*s1m1 and any variant of the *cagA* gene or *cag*PAI were not associated with diseases in India[51,71-73]. However, colonization by multiple *H. pylori* strains in single gastric mucosa is fairly common and single gastric biopsy based culture do not represent all the strains that colonize in a stomach. Infection with strains with *vacA*s1m1 and the *cagA* has been found in individual, who is also infected with strain carrying *vacA*s2m2 and lacks *cag*PAI[74]. At present we do not understand the significance of this association among multiple *H. pylori* strains (Figure 1). But, since *H. pylori* is a genetically diverse species that is naturally competent and capable of horizontal gene transfer, the presence of multiple *H. pylori* strains with different genotypes may confer some adaptive advantages, like overcoming immune responses. How the immune surveillance varies with host genetic background and how it affects clinical outcomes in relation to *H. pylori* colonization? Besides, the VacA and the CagA proteins, although are the most studied, are not the only virulence markers for *H. pylori* (eg. NapA, OipA, BabA, IceA, DupA)[18]. How the interactions among these virulence markers actually contribute in the progression of gastroduodenal diseases as well extragastric diseases like neurodegenerative disorders? *H. pylori* can alter the function of tight junctions but can it actually modulate the gut-brain axis[75]? If an individual is infected with *H. pylori* but does not have ulcer or cancer, should the individual be treated with antibiotics in order to prevent these diseases? Intriguingly, the eradication of *H. pylori* is also associated with esophageal cancer[6]. Moreover, antibiotic treatment changes the dynamics of the commensal gut flora and may lead to increase in number of the opportunistic pathobionts, like *Clostridium difficile*[5]. Several lytic phages for *H. pylori* have been reported[76,77]. Is it possible to specifically cure *H. pylori* using lytic phages without affecting the microbiota? While several arguments suggested that *H. pylori* is indigenous microbiota of humans, the roles of VacA and CagA in the progression of gastroduodenal illness have been confirmed by numerous studies[70,78]. Therefore, the most interesting question is, how ~80% of the infected population can make a feasible relationship with *H. pylori* (with all strains?), while others suffer from similar infection? Likewise, why everybody among the uninfected population (~50%) is not suffering from esophageal cancer and asthma?

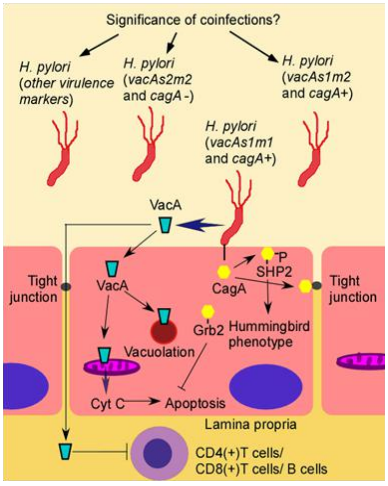

Figure 1. Cartoon showing the functions of the VacA and the CagA of *H. pylori*. The VacA is a secreted toxin, which gets internalized through receptors. Inside the cell, the VacA forms vacuoles and interacts with cellular proteins to regulate functions like induction of apoptosis and suppression of immunity. The CagA is injected into the cell by the type IV secretion system. Inside cytoplasm, the CagA is phosphorylated and binds to SHP2, which leads to hummingbird phenotype and cancer. The unphosphorylated CagA also binds to many proteins including Grb2. The CagA-Grb2 complex formation leads to a cascade of events, which leads to inhibition of apoptosis. The CagA can also be associated with the tight junction proteins. The significance of multiple infections with different genotype carrying strains is not well documented.

In relation to *H. pylori* infections, we still have more questions than answers (Table 1). Recent literature show the promise of unraveling many of these issues, but our understanding of *H. pylori* infected gastric milieu is reasonably inadequate at present. A multidisciplinary interactive approach involving metagenomics, proteomics, immunology and genetics is needed to understand the mechanisms that regulate clinical outcomes in relation to *H. pylori* infections.

Table 1. Some important questions in relation to *H. pylori* infection

• Why the expression of CagA is highly correlated with the expression of VacA?
• What are the combined roles of *H. pylori* virulence markers?
• Does having multiple genotypes in same niche help *H. pylori* to evade immune surveillance?
• How the immune surveillance varies with host genetic background?
• Can *H. pylori* modulate the gut-brain axis?
• Should every *H. pylori* infected person be treated with antibiotics?
• Can lytic phages be used for the treatment of *H. pylori* infections?
• How ~80% of the *H. pylori* infected individuals tolerate the infection?

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References

1. Marshall, B.J., Warren, J.R. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. (1984) Lancet 1(8390): 1311- 5.
2. Wotherspoon, A.C., Ortiz-Hidalgo, C., Falzon, M.R., et al. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. (1991) Lancet 338(8776): 1175- 6.
3. Malfertheiner, P., Link, A., Selgrad, M. Helicobacter pylori: perspec-

tives and time trends. (2014) Nat Rev Gastroenterol Hepatol. 11(10): 628- 38.
4. Malfertheiner, P., Megraud, F., O'Morain, C.A., et al. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. (2012) Gut 61(5): 646- 64.
5. Malnick, S.D., Melzer, E., Attali, M., et al. Helicobacter pylori: friend or foe? (2014) World J Gastroenterol. 20(27): 8979- 85.
6. Blaser, M.J. Disappearing microbiota: Helicobacter pylori protection against esophageal adenocarcinoma. (2008) Cancer Prev Res (Phila). 1(5): 308- 11.
7. Leunk, R.D., Johnson, P.T., David, B.C., et al. Cytotoxic activity in broth-culture filtrates of Campylobacter pylori. J Med Microbiol. (1988) 26(2): 93- 9.
8. Cover, T.L., Blaser, M.J. Purification and characterization of the vacuolating toxin from Helicobacter pylori. (1992) J Biol Chem. 267(15): 10570- 5.
9. Cover, T.L., Tummuru, M.K., Cao, P., et al. Divergence of genetic sequences for the vacuolating cytotoxin among Helicobacter pylori strains. (1994) J Biol Chem 269(14): 10566-73.
10. Palframan, S.L., Kwok, T., Gabriel, K. Vacuolating cytotoxin A (VacA), a key toxin for Helicobacter pylori pathogenesis. (2012) Front Cell Infect Microbiol. 2: 92.
11. Atherton, J.C., Cao, P., Peek, R.M., et al. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. (1995) J Biol Chem. 270(30): 17771- 7.
12. Letley, D.P., Atherton, J.C. Natural diversity in the N terminus of the mature vacuolating cytotoxin of Helicobacter pylori determines cytotoxin activity. (2000) J Bacteriol. 182(11): 3278- 80.
13. McClain, M.S., Cao, P., Iwamoto, H., et al. A 12-amino-acid segment, present in type s2 but not type s1 Helicobacter pylori VacA proteins, abolishes cytotoxin activity and alters membrane channel formation. (2001) J Bacteriol. 183(22): 6499- 508.
14. Atherton, J.C., Peek, R.M. Jr., Tham, K.T., et al. Clinical and pathological importance of heterogeneity in vacA, the vacuolating cytotoxin gene of Helicobacter pylori. (1997) Gastroenterology. 112(1): 92- 9.
15. Ji, X., Fernandez, T., Burroni, D., et al. Cell specificity of Helicobacter pylori cytotoxin is determined by a short region in the polymorphic midregion. (2000) Infect Immun. 68(6): 3754- 7.
16. Skibinski, D.A., Genisset, C., Barone, S., et al. The cell-specific phenotype of the polymorphic vacA midregion is independent of the appearance of the cell surface receptor protein tyrosine phosphatase beta. (2006) Infect Immun. 74(1): 49- 55.
17. Pagliaccia, C., de Bernard, M., Lupetti, P., et al. The m2 form of the Helicobacter pylori cytotoxin has cell type-specific vacuolating activity. (1998) Proc Natl Acad Sci U S A. 95(17): 10212- 7.
18. Yamaoka, Y. Mechanisms of disease: Helicobacter pylori virulence factors. (2010) Nat Rev Gastroenterol Hepatol. 7(11): 629- 41.
19. Rhead, J.L., Letley, D.P., Mohammadi, M., et al. A new Helicobacter pylori vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. (2007) Gastroenterology. 133(3): 926- 36.
20. Chung, C., Olivares, A., Torres, E., et al. Diversity of VacA intermediate region among Helicobacter pylori strains from several regions of the world. (2010) J Clin Microbiol. 48(3): 690- 6.
21. Basso, D., Zambon, C.F., Letley, D.P., et al. Clinical relevance of Helicobacter pylori cagA and vacA gene polymorphisms. (2008) Gastroenterology. 135(1): 91- 9.
22. Schmitt, W., Haas, R. Genetic analysis of the Helicobacter pylori vacuolating cytotoxin: structural similarities with the IgA protease type of exported protein. (1994) Mol Microbiol. 12(2): 307- 19.
23. Cover, T.L., Hanson, P.I., Heuser, J.E. Acid-induced dissociation of VacA, the Helicobacter pylori vacuolating cytotoxin, reveals its pattern of assembly. (1997) J Cell Biol. 138(4): 759- 69.
24. Kim, I.J., Blanke, S.R. Remodeling the host environment: modulation of the gastric epithelium by the Helicobacter pylori vacuolating toxin (VacA). (2012) Front Cell Infect Microbiol 2: 37.
25. de Bernard, M., Papini, E., de Filippis, V., et al. Low pH activates the vacuolating toxin of Helicobacter pylori, which becomes acid and pepsin resistant. (1995) J Biol Chem. 270(41): 23937- 40.
26. Gangwer, K.A., Mushrush, D.J., Stauff, D.L., et al. Crystal structure of the Helicobacter pylori vacuolating toxin p55 domain. (2007) Proc Natl Acad Sci U S A. 104(41): 16293- 8.
27. Torres, V.J., Ivie, S.E., McClain, M.S., et al. Functional properties of the p33 and p55 domains of the Helicobacter pylori vacuolating cytotoxin. (2005) J Biol Chem. 280(22): 21107-14.
28. Sewald, X., Fischer, W., Haas, R. Sticky socks: Helicobacter pylori VacA takes shape. (2008) Trends Microbiol. 16(3): 89- 92.
29. Cover, T.L., Puryear, W., Perez-Perez, G.I., et al. Effect of urease on HeLa cell vacuolation induced by Helicobacter pylori cytotoxin. (1991) Infect Immun. 59(4): 1264- 70.
30. Papini, E., de Bernard, M., Milia, E., et al. Cellular vacuoles induced by Helicobacter pylori originate from late endosomal compartments. (1994) Proc Natl Acad Sci U S A. 91(21): 9720- 4.
31. Papini, E., Gottardi, E., Satin, B., et al. The vacuolar ATPase proton pump is present on intracellular vacuoles induced by Helicobacter pylori. (1996) J Med Microbiol. 45(2): 84- 9.
32. de Bernard, M., Burroni, D., Papini, E. Identification of the Helicobacter pylori VacA toxin domain active in the cell cytosol. (1998) Infect Immun. 66(12): 6014- 6.
33. Isomoto, H., Moss, J., Hirayama, T. Pleiotropic actions of Helicobacter pylori vacuolating cytotoxin, VacA. (2010) Tohoku J Exp Med. 220(1): 3- 14.
34. Yamasaki, E., Wada, A., Kumatori, A., et al. Helicobacter pylori vacuolating cytotoxin induces activation of the proapoptotic proteins Bax and Bak, leading to cytochrome c release and cell death, independent of vacuolation. (2006) J Biol Chem. 281(16): 11250- 9.
35. Willhite, D.C., Blanke, S.R. Helicobacter pylori vacuolating cytotoxin enters cells, localizes to the mitochondria, and induces mitochondrial membrane permeability changes correlated to toxin channel activity. (2004) Cell Microbiol. 6(2): 143- 54.
36. Manente, L., Perna, A., Buommino, E., et al. The Helicobacter pylori's protein VacA has direct effects on the regulation of cell cycle and apoptosis in gastric epithelial cells. (2008) J Cell Physiol. 214(3): 582- 7.
37. Radin, J.N., Gonzalez-Rivera, C., Ivie, S.E., et al. Helicobacter pylori VacA induces programmed necrosis in gastric epithelial cells. (2011) Infect Immun. 79(7): 2535- 43.
38. Yahiro, K., Satoh, M., Nakano, M., et al. Low-density lipoprotein receptor-related protein-1 (LRP1) mediates autophagy and apoptosis caused by Helicobacter pylori VacA. (2012) J Biol Chem. 287(37): 31104- 15.
39. Nakayama, M., Kimura, M., Wada, A., et al. Helicobacter pylori VacA activates the p38/activating transcription factor 2-mediated signal pathway in AZ-521 cells. (2004) J Biol Chem. 279(8): 7024- 8.
40. Nakayama, M., Hisatsune, J., Yamasaki, E., et al. Helicobacter pylori VacA-induced inhibition of GSK3 through the PI3K/Akt signaling pathway. (2009) J Biol Chem. 284(3): 1612- 9.
41. Gebert, B., Fischer, W., Weiss, E., et al. Helicobacter pylori vacuolating cytotoxin inhibits T lymphocyte activation. (2003) Science 301(5636): 1099- 102.
42. Torres, V.J., VanCompernolle, S.E., Sundrud, M.S., et al. Helicobacter pylori vacuolating cytotoxin inhibits activation-induced proliferation of human T and B lymphocyte subsets. (2007) J Immunol. 179(8): 5433- 40.
43. Apel, I., Jacobs, E., Kist, M., et al. Antibody response of patients against a 120 kDa surface protein of Campylobacter pylori. (1988) Zentralbl Bakteriol Mikrobiol Hyg A. 268(2): 271- 6.
44. Cover, T.L., Dooley, C.P., Blaser, M.J. Characterization of and human serologic response to proteins in Helicobacter pylori broth culture supernatants with vacuolizing cytotoxin activity. (1990) Infect Immun. 58(3): 603- 10.
45. Tummuru, M.K., Cover, T.L., Blaser, M.J. Cloning and expression of a high-molecular-mass major antigen of Helicobacter pylori: evidence of linkage to cytotoxin production. (1993) Infect Immun. 61(5): 1799- 809.

46. Covacci, A., Censini, S., Bugnoli, M., et al. Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. (1993) Proc Natl Acad Sci U S A. 90(12): 5791- 5.
47. Censini, S., Lange, C., Xiang, Z. et al. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. (1996) Proc Natl Acad Sci U S A. 93(25): 14648- 53.
48. Mukhopadhyay, A.K., Kersulyte, D., Jeong, J.Y., et al. Distinctiveness of genotypes of Helicobacter pylori in Calcutta, India. (2000) J Bacteriol. 182(11): 3219- 27.
49. Hatakeyama, M. Anthropological and clinical implications for the structural diversity of the Helicobacter pylori CagA oncoprotein. (2011) Cancer Sci. 102(1): 36- 43.
50. Higashi, H., Tsutsumi, R., Fujita, A. et al. Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. (2002) Proc Natl Acad Sci U S A. 99(22): 14428- 33.
51. Chattopadhyay, S., Patra, R., Chatterjee, R., et al. Distinct repeat motifs at the C-terminal region of CagA of Helicobacter pylori strains isolated from diseased patients and asymptomatic individuals in West Bengal, India. (2012) Gut Pathog 4(1): 4.
52. Yamaoka, Y., Kodama, T., Kashima, K., et al. Variants of the 3' region of the cagA gene in Helicobacter pylori isolates from patients with different H. pylori-associated diseases. (1998) J Clin Microbiol 36(8): 2258- 63.
53. Furuta, Y., Yahara, K., Hatakeyama, M., et al. Evolution of cagA oncogene of Helicobacter pylori through recombination. (2011) PLoS One 6(8): e23499.
54. Hayashi, T., Senda, M., Morohashi, H., et al. Tertiary structure-function analysis reveals the pathogenic signaling potentiation mechanism of Helicobacter pylori oncogenic effector CagA. (2012) Cell Host Microbe. 12(1): 20- 33.
55. Kaplan-Turkoz, B., Jimenez-Soto, L.F., Dian, C., et al. Structural insights into Helicobacter pylori oncoprotein CagA interaction with beta1 integrin. (2012) Proc Natl Acad Sci U S A. 109(36): 14640- 5.
56. Woon, A.P., Tohidpour, A., Alonso, H., et al. Conformational analysis of isolated domains of Helicobacter pylori CagA. (2013) PLoS One 8(11): e79367.
57. Ren, S., Higashi, H., Lu, H., et al. Structural basis and functional consequence of Helicobacter pylori CagA multimerization in cells. (2006) J Biol Chem. 281(43): 32344- 52.
58. Jimenez-Soto, L.F., Kutter, S., Sewald, X., et al. Helicobacter pylori type IV secretion apparatus exploits beta1 integrin in a novel RGD-independent manner. (2009) PLoS Pathog. 5(12): e1000684.
59. Stein, M., Bagnoli, F., Halenbeck, R., et al. c-Src/Lyn kinases activate Helicobacter pylori CagA through tyrosine phosphorylation of the EPIYA motifs. (2002) Mol Microbiol 43(4): 971- 80.
60. Higashi, H., Tsutsumi, R., Muto, S., et al. SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. (2002) Science 295(5555): 683- 6.
61. Azuma, T., Yamazaki, S., Yamakawa, A., et al. Association between diversity in the Src homology 2 domain--containing tyrosine phosphatase binding site of Helicobacter pylori CagA protein and gastric atrophy and cancer. (2004) J Infect Dis. 189(5): 820- 7.
62. Ohnishi, N., Yuasa, H., Tanaka, S., et al. Transgenic expression of Helicobacter pylori CagA induces gastrointestinal and hematopoietic neoplasms in mouse. (2008) Proc Natl Acad Sci U S A. 105(3): 1003- 8.
63. Tsutsumi, R., Higashi, H., Higuchi, M., et al. Attenuation of Helicobacter pylori CagA x SHP-2 signaling by interaction between CagA and C-terminal Src kinase. (2003) J Biol Chem 278(6): 3664- 70.
64. Selbach, M., Paul, F.E., Brandt, S., et al. Host cell interactome of tyrosine-phosphorylated bacterial proteins. (2009) Cell Host Microbe 5(4): 397- 403.
65. Mimuro, H., Suzuki, T., Tanaka, J., et al. Grb2 is a key mediator of helicobacter pylori CagA protein activities. (2002) Mol Cell 10(4): 745- 55.
66. Mimuro, H., Suzuki, T., Nagai, S., et al. Helicobacter pylori dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. (2007) Cell Host Microbe 2(4): 250- 63.
67. Song, X., Chen, H.X., Wang, X.Y., et al. H. pylori-encoded CagA disrupts tight junctions and induces invasiveness of AGS gastric carcinoma cells via Cdx2-dependent targeting of Claudin-2. (2013) Cell Immunol 286(1- 2): 22- 30.
68. Amieva, M.R., Vogelmann, R., Covacci, A., et al. Disruption of the epithelial apical-junctional complex by Helicobacter pylori CagA. (2003) Science 300(5624): 1430- 4.
69. Handa, O., Naito, Y., Yoshikawa, T. CagA protein of Helicobacter pylori: a hijacker of gastric epithelial cell signaling. (2007) Biochem Pharmacol 73(11): 1697- 702.
70. Jones, K.R., Whitmire, J.M., Merrell, D.S. A. Tale of Two Toxins: Helicobacter Pylori CagA and VacA Modulate Host Pathways that Impact Disease. (2010) Front Microbiol 1: 115.
71. Chattopadhyay, S., Datta, S., Chowdhury, A., et al. Virulence genes in Helicobacter pylori strains from West Bengal residents with overt H. pylori-associated disease and healthy volunteers. (2002) J Clin Microbiol 40(7): 2622- 5.
72. Datta, S., Chattopadhyay, S., Balakrish, N. G., et al. Virulence genes and neutral DNA markers of Helicobacter pylori isolates from different ethnic communities of West Bengal, India. (2003) J Clin Microbiol 41(8): 3737- 43.
73. Patra, R., Chattopadhyay, S., De, R., et al. Intact cag pathogenicity island of Helicobacter pylori without disease association in Kolkata, India. (2011) Int J Med Microbiol 301(4): 293- 302.
74. Patra, R., Chattopadhyay, S., De, R., et al. Multiple infection and microdiversity among Helicobacter pylori isolates in a single host in India. (2012) PLoS One 7(8): e43370.
75. Budzynski, J., Klopocka, M. Brain-gut axis in the pathogenesis of Helicobacter pylori infection. (2014) World J Gastroenterol 20(18): 5212- 25.
76. Luo, C.H., Chiou, P.Y., Yang, C.Y., et al. Genome, integration, and transduction of a novel temperate phage of Helicobacter pylori. (2012) J Virol 86(16): 8781- 92.
77. Uchiyama, J., Takeuchi, H., Kato, S., et al. Characterization of Helicobacter pylori bacteriophage KHP30. (2013) Appl Environ Microbiol 79(10): 3176- 84.
78. Blaser, M.J., Falkow, S. What are the consequences of the disappearing human microbiota? (2009) Nat Rev Microbiol 7(12): 887- 94.