The Mechanism of CagA and VacA in Gastric Cancer under the Tumor Microenvironment and Vitro Factors

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Abstract: Gastric cancer is closely related to the stomach microbiota, especially *Helicobacter pylori*. Numerous reports and clinical studies have shown that microbial behavior in the stomach may lead to pathological changes in the gastrointestinal tract of the host, which ultimately leads to the production and development of gastric cancer. This review outlines the major pathogenic processes of *Helicobacter pylori* in the stomach, specifically focusing on CagA, VacA, inflammatory pathways and oxidative stress. In addition, we describe the effects of some non-*Helicobacter pylori* factors, such as other microbiota, alcohol, and tobacco, on the carcinogenesis induced by *Helicobacter pylori*. The effects of family history are also taken into account. We hope that understanding the stomach microbiota will make it possible to more easily prevent, detect and treat gastric cancer.

Key words: Microbiota; Gastric cancer; *Helicobacter pylori*; CagA; VacA; Inflammatory pathway

Introduction

Gastric cancer (GC) is the fourth most common cancer and the second leading cause of cancer death worldwide [1]. About half of all cases and deaths due to GC occur in China, which causes a heavy burden on the Chinese healthcare system [2]. The stomach has been considered sterile, but in fact contains a variety of bacterial communities [3]. The best-known bacterium is *Helicobacter pylori* (*H. pylori*). In many areas of the world, including various parts of Asia, *H. pylori* infection rates are directly correlated with the rates of GC. [4]. However, while it has been known for several decades that *H. pylori* is related to GC [5,6], the mechanisms by which it induces carcinogenesis, and other factors that regulate the response to *H. pylori* infection, are still being uncovered.

We herein review recent research on the stomach microbiome and the mechanisms underlying *H. pylori*-induced carcinogenesis. These studies clearly demonstrate the pathological effects of certain microbial communities on the stomach [7]. As is well-known, *H. pylori* causes a variety of pathological changes in the host stomach, including chronic gastritis, atrophy, intestinal metaplasia and GC [8]. This review describes the recent findings regarding the major pathogenic processes associated

with *H. pylori* in the stomach, focusing on cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA), inflammatory pathways and oxidative stress. We also describe the effects of some non-*Helicobacter pylori* factors, such as other microbes, alcohol, and tobacco, on the induction of carcinogenesis by *H. pylori*. In addition, we take into account the effects of family history, and explore the impact of the host's genetics on the *H. pylori*-mediated carcinogenesis. As more information becomes available about the stomach microbiota, we hope that it will become possible to prevent, detect and treat gastric cancer by using more advanced methods for gastric cancer screening and via microbial regulation.

The Carcinogenic Effects of *H. pylori* in the Human Stomach

The highly acidic environment in the stomach is lethal to many species of bacteria and parasites. However, there are others that have adapted to survive under such harsh conditions, including *H. pylori*. *H. pylori* has become symbiotic with humans [9], gradually evolving the ability to settle and survive in the highly acidic stomach of the human body [10]. Examples of such adaptations by *H. pylori* include the ability to change their shape and the number of flagella to maintain motility and chemotaxis in a viscous environment [11,12]. The bacteria also produce urease and other factors that help them adapt to the acidic conditions in the lumen [13] and mediate the preferential colonization of *H. pylori* at sites of gastric injury [14].

CagA

The cag pathogenicity island is a special fragment present in a 40 kb DNA sequence that encodes the CagA

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protein and the type IV secretion system (T4SS) [15,16]. CagA is injected into the cytoplasm of gastric epithelial cells by *H. pylori* through the T4SS structure [17]. It has been demonstrated that CagA is a key factor involved in human chronic gastritis and ulcers, mucosa-associated lymphoid tissue lymphoma and GC [18]. Additionally, studies have shown that CagA-positive strains of bacteria are associated with a several-fold increase in the risk of GC compared with CagA-negative strains [19]. A meta-analysis further confirmed that individuals infected with CagA-positive *H. pylori* strains had an increased risk of developing GC [20,21].

CagA is a macromolecular cytoplasmic protein produced. Due to the structural differences in the C-terminal region, the molecule is subject to change [22], ranging from between about 120 and 140 kD in length. CagA has a tyrosine phosphorylated region and a non-phosphorylated region [23]. Interestingly, the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif present in the C-terminal region is a target for phosphorylation by Src family kinase (SFK) [24] and Abl families [25], while other regions are unable to undergo phosphorylation. CagA binds to various signaling proteins and causes dysregulation of multiple signaling pathways in a phosphorylation-dependent or -independent manner, inducing cancer via these pathways [26].

Tyrosine Phosphorylation

After tyrosine phosphorylation, segments C and D of EPIYA (EPIYA has four segments A, B, C, and D [27]) acquire the ability to bind to the Src-homology-2 domain containing PTPase (SHP2) [28] (Figure 1). SHP2 is a tyrosine phosphorylase that plays an important role in cell mitosis [29]. Although it has been suggested that East Asian CagA and Western CagA use different mechanisms to deregulate SHP2 [30], CagA binds to SHP2, activates SHP2 activity [28], enhances Ras-extracellular regulated protein kinases (Erk) signaling [31], and promotes abnormal mitosis. Studies have also shown that SHP2 is a proto-oncogene phosphatase [32], suggesting a link between CagA and GC. Researchers have shown that CagA interacts with C10 regulator of kinase (Crk) to play an important role in the reduction of gastric epithelial cell adhesion caused by *H. pylori* [33]. Crk-induced Src activation and the subsequent signaling of p38 mitogen-activated protein kinase (MAPK) promote the proliferation of sarcoma cells [34]. There is also an interaction between phosphorylated CagA and CagA-c-terminal Src kinase (Csk) [35] that leads to cell elongation called the “hummingbird phenotype” [36]. Csk phosphorylation inhibits SFK kinase activity, resulting in decreased EPIYA phosphorylation of CagA, which can effectively prevent tyrosine phosphorylation-dependent

Figure 1 The CagA-positive *H. pylori* tyrosine phosphorylation pathway. (a) SHP2 acts as a tyrosine phosphorylase and proto-oncogene phosphorylase. It binds to CagA, enhances Erk signaling, and promotes abnormal mitosis. (b) CagA interacts with Crk to reduce the adhesion of gastric epithelial cells. Crk can induce the Src and MAPK pathways, promoting the proliferation of sarcoma cells. (c) Phosphorylated CagA can also interact with Csk to induce a “hummingbird phenotype” in cells. At the same time, Csk’s inhibition of SFK is weakened, which will increase the risk of cancer.

overactivation of CagA activity. Although this has been suggested to have inhibitory effects on *H. pylori* [37], the bacterium remains closely associated with the development of GC.

Non-Tyrosine Phosphorylation

In the absence of tyrosine phosphorylation, there is dysregulation of various signaling pathways. In the phosphorylation-independent pathway, unphosphorylated CagA interacts with various signaling proteins, such as hepatocyte growth factor receptor (c-met), E-cadherin [38], growth factor receptor-bound 2 (Grb2) [39], and partitioning-defective 1b (Par1b) [40], and then activates the corresponding signaling pathway (Figure 2). *H. pylori* CagA targets the c-met receptor and enhances the cell motility. During the translocation process, CagA regulates cellular

function by releasing the c-met receptor signal [41].

The occurrence of GC is inseparable from the process of inflammation. The CagA protein negatively regulates autophagy and promotes an inflammatory response to *H. pylori* infection. This inflammatory response is regulated by activation of the c-met-phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)-mammalian target of rapamycin (mTOR) signaling pathway [38]. The PI3K/Akt signaling pathway is strongly activated in GC [42].

H. pylori CagA also interacts with E-cadherin to promote beta-catenin signaling and the intestinal metaplasia of gastric epithelial cells [43], a precancerous lesion of the stomach. CagA interacts with Grb2, which activates the Ras/MEK/ERK pathway, leading to cell proliferation [39]. The interaction between CagA and Par1b inhibits Par1b kinase activity, affects the actin cytoskeletal system, and

Figure 2 The CagA-positive *H. pylori* non-tyrosine phosphorylation pathway. (a) CagA binds to c-met, releasing receptor signals to promote bacterial translocation. At the same time, the c-met pathway activates the downstream PI3K-Akt-mTOR pathway and participates in the inflammatory response. (b) CagA interacts with E-cadherin to induce β -catenin signaling and promote the intestinal metaplasia of gastric epithelial cells. (c) CagA interacts with Grb2 to activate Ras/MEK/ERK, resulting in cell proliferation. (d) The actions of CagA and Par1b inhibit Par1b kinase activity and enhances the “hummingbird phenotype”. (e) CagA activates the highly conserved Wnt/ β -catenin pathway and promotes gastric cancer stem cell activity. (f) CagA induces NF- κ B, decreases the expression of E-cadherin, and participates in tumor invasion. (g) CagA induces the JNK pathway and activates the STAT3-Akt signaling axis. At the same time, CagA inhibits p53 and induces RUNX3 methylation, leading to an increased risk of gastric cancer. In each of these processes, the nucleus is involved in the regulation of cellular activities.

enhances the size of cells with the hummingbird phenotype [40]. Neal JT et al. established a transgenic zebrafish model to study CagA, and found that it increases intestinal cell proliferation through activation of the Wnt pathway, leading to GC [44]. In general, the Wnt/ β -catenin signaling pathway is highly conserved among species. Thus, *H. pylori* CagA-positive strains can promote the activity of human GC stem cells through the Wnt/ β -catenin signaling pathway [45,46].

Kim SY et al. used eukaryotic expression vectors and showed that CagA induced NF- κ B (NF- κ B) activation and IL-8 release [47]. The NF- κ B pathway is involved in tumor invasion and metastasis by reducing the expression of E-cadherin [48]. Recently, Wandler AM et al. used the JNK (c-Jun NH₂-terminal kinase) pathway activation in a *Drosophila* model to demonstrate that CagA acts as an important mediator of activation of this signaling pathway [49,50], which ultimately promotes apoptosis or tumorigenesis. Further studies have found that there may be a carcinogenic pathway linking the JNK-STAT3-Akt signaling axis [51]. *H. pylori* CagA was shown to promote the development of GC through activation of gastric STAT3 [52].

Interestingly, CagA can also interfere with certain tumor suppressors [53]. The experimental results described by Wei J et al. provided new evidence that the tumorigenicity associated with *H. pylori* infection is associated with CagA-mediated inhibition of p53 protein [54]. CagA enhances the degradation of p53 and down-regulates its activity [55]. Another study found that RUNX3 methylation was increased in poorly differentiated adenocarcinoma [56], thus decreasing the transcriptional activation of RUNX3 [57], thereby increasing the risk of GC. CagA has been drawing increasing attention in recent years, so it is possible that additional CagA-related effects will be discovered in the near future.

VacA

VacA is a vacuolar cytotoxin isolated from the supernatant of *H. pylori* culture medium, and is also a secreted toxin. VacA toxin is an 88 kDa pathogenic factor that consists of two domains; p33 and p55 [58]. Torres VJ et al. demonstrated that these two domains can interact to form a protein complex, and the binding of the mixture to the plasma membrane of mammalian cells is significantly enhanced compared to the binding of the VacA domain alone [59,60]. After entering eukaryotic cells, VacA leads to cell vacuolization [61]. *H. pylori* VacA forms an anion-selective channel in the planar lipid bilayer, which may induce a permeability imbalance in the intracellular acidic compartment [62]. Czajkowsky DM et al. found that when the pH is below 5, VacA combines with the anionic lipid bilayer to form a hexa-membrane-related complex, thereby forming pores [63]. This low pH-triggered pore formation may be a critical step in VacA activity [64], and the acidic environment of the stomach is conducive to this activity.

VacA has a variety of effects on cells, although the most important are altering the permeability of the plasma membrane and causing vacuolation of epithelial cells [65,66]. VacA induces autophagy, but the mechanism underlying this finding remains unclear [67]. Raju D et al. added VacA components to cultured gastric epithelial cells, and found that VacA could induce autophagy and regulate the degradation and circulation of cellular components in the cytoplasm [68]. VacA is also involved in biological reactions in the mitochondria. Jain P et al. found that *H. pylori* interfered with the morphological dynamics of mitochondria [69]. Chatre L et al. also suggested that *H. pylori* induces mitochondrial replication [70]. These changes are consistent with the progression of gastric lesions.

VacA also has immunomodulatory and inhibitory activities on various cells of the mammalian immune system, ensuring the persistence of the host *H. pylori* [71]. VacA can inhibit the activation and proliferation of T cells and B cells, and can interfere with the antigen expression of B cells [72]. It also prevents the normal maturation, antigen processing and expression of phagocytic cells, and interferes with intracellular killing and cytokine production [73]. The immune system is thus inhibited, ensuring continued infection with *H. pylori*.

VacA is similar to CagA with regard to its effects on gastric acid. Wang F et al. used freshly isolated rabbit gastric glands and cultured parietal cells to study the effects of VacA infection on the physiological function of parietal cells, and found that VacA penetrated into the apical membrane of these cells and induced hypochlorite production [74]. These effects echoed the earlier findings by Kobayashi's team showing that VacA affected gastric acid secretion in guinea pig isolated wall cells [75]. Animal models have also revealed that VacA plays a role in promoting gastric pathology [76,77]. For example, VacA can cause damage to the gastric mucosa, increase inflammatory cells, and induce cancer [78].

The potential interaction of CagA and VacA is not well understood. However, VacA stimulates CagA degradation through an autophagy pathway, thereby reducing the half-life of CagA [79,80]. However, in gastric cells expressing CD44v9, autophagy is prevented, allowing the accumulation of CagA [81]. Therefore, the probability of cancer increases in cells that are invaded by *H. pylori*. In addition to CagA and VacA, genes such as *iceA* and *babA2* also play a role in GC [82,83], mainly due to the increased risk of *H. pylori*-related diseases [84]. Moreover, there is a geographical dependence in terms of the roles and importance of the different gene products, providing some clues to the pathogenesis of *H. pylori*, and these agents are often closely related to the clinical outcomes [85].

Inflammatory Response and Oxidative Stress Process

The human immune system has innate and adaptive

immune responses to *H. pylori* [86], which can chronically proliferate and actively adapt to unfavorable parts of the stomach, making it difficult to eradicate [87]. Therefore, infection with *H. pylori* often leads to chronic inflammation, and oxidative stress may be caused by the inflammation. Although infected patients may remain asymptomatic indefinitely, *H. pylori* can induce an inflammatory response in gastric epithelial cells and may lead to the circulation of immune cells through various routes. Activation of NF- κ B and up-regulation of IL-8 in gastric epithelial cells

are considered to be critical for both *H. pylori*-induced chronic inflammation and GC [88,89]. Like the microbes associated with colorectal cancer-induced inflammation, *H. pylori* activates NF- κ B by binding to the toll-like receptors (TLR) of epithelial cells and binding to myeloid differentiation factor 88 (MyD88) [90] (Figure 3). Lamb A et al. demonstrated that CagA and transforming growth factor- β -activated kinase 1 (TAK1) are essential for *H. pylori* to induce NF- κ B activation [91]. CagA induction is mediated by tumor necrosis factor receptor-associated

Figure 3 This image shows the carcinogenic process associated with *H. pylori*-related inflammation and oxidative stress. In the inflammatory response, *H. pylori* binds to MyD88 via TLRs and activates NF- κ B. NF- κ B activation activates the COX-2 and PGE-2 pathways and induces the release of cytokines such as TNF- α and IL-11, leading to gastric cancer. *H. pylori* releases CagA through T4SS. CagA stimulation leads to an increase in the SMO levels in the gastric epithelium and produces H₂O₂, leading to oxidative stress. H₂O₂ depolarizes the mitochondria and induces ROS production. The accumulation of ROS also depends on the toxin inhibiting the GSH pathway. ROS not only cause nuclear DNA damage, but also induce the up-regulation of IL-6, activate STAT3-NF- κ B, thus promoting the activation of proto-oncogenes. ROS can cause apoptosis through cytochromes. CagA also binds to TAK1 and induces NF- κ B activation mediated by TRAF6. CagA-dependent NF- κ B activation can also be mediated through the PI3K/Akt signaling pathway. CagA negatively regulated autophagy and inhibits the anti-inflammatory effects of HO-1, leading to inflammation. VacA activates MAPK and NF- κ B by releasing calcium ions. VacA also causes morphological changes in mitochondria, inhibiting gastric acid secretion and immune cell function. *H. pylori* can lead to the development of gastric cancer through all of these pathways.

factor 6 (TRAF6), which binds to TAK1 to induce NF- κ B activation. CagA-dependent NF- κ B activation can also be mediated through the PI3K/Akt signaling pathway [92]. The development of GC is considered to involve the combination of inflammation and oncogenes. Most tumors can induce cyclo-oxygenase (COX-2) expression, and NF- κ B activation further increases the activity of the COX-2 and prostaglandin E2 (PGE-2) pathways. PGE-2 binds to the EP4 receptor [93], activates MAPK signaling [94], and induces the release of cytokines such as IL-11 and TNF- α . COX-2 also regulates E-cadherin expression via the NF- κ B signaling pathway [48], which plays a role in the CagA oncogenic pathway.

In addition to the inflammatory response, the oxidative stress induced by *H. pylori* cannot be ignored. Chaturvedi R et al. recently reported that *H. pylori* expressing CagA leads to elevated levels of spermine oxidase (SMO) in gastric epithelial cells [95]. SMO decomposes polyamine spermine, which produces hydrogen peroxide (H_2O_2), leading to apoptosis and DNA damage. *H. pylori* CagA also oxidizes DNA by inducing SMO. These subpopulations of cells are often resistant to apoptosis, and therefore have a high risk of malignant transformation [96]. Hydrogen peroxide can depolarize mitochondria [97] and induce reactive oxygen species (ROS), which will continue to react with the nucleus [98]. ROS not only cause DNA damage, but also induce the upregulation of IL-6, which mediates STAT3 activation [99]. The STAT3 and NF- κ B pathways are inextricably linked to the activation of proto-oncogenes, providing a potential link between inflammation and GC [100]. Hryciak EG et al. also found that ROS have effects on cytochromes in gastric epithelial cells and can induce apoptosis [101]. Heme oxygenase-1 (HO-1) has antioxidant and anti-inflammatory effects [102].

Interestingly, *H. pylori* CagA has been suggested to reduce the incidence of GC by destroying nitric oxide, reducing NF- κ B activation, inhibiting heme oxygenase-1, and enhancing the inflammatory response [103]. CagA negatively regulates autophagy and promotes inflammation [104]. VacA activates p38 MAPK by inducing the release of intracellular calcium ions, directly increasing the production of IL-8, thereby activating NF- κ B [105]. VacA is a chloride channel that targets mitochondria [106]. Recent studies have provided new perspectives regarding VacA-induced loss of the mitochondrial membrane potential, mitochondrial fragmentation, reactive oxygen species formation, autophagy [107], and the mechanism underlying the development of GC. Interestingly, after further studies, Tsugawa H et al. found that CagA accumulation in gastric cells expressing CD44 is due to VacA causing ROS accumulation and Akt activation by decreasing the intracellular glutathione (GSH) levels [80]. CD44 is a cell surface marker associated with cancer stem cells [108]. Together, these findings provide a possible molecular link between *H. pylori* and gastric carcinogenesis.

Carcinogenesis Related to Non-*H. pylori* Factors in the Human Stomach

Although *H. pylori* infection is considered to be a major risk factor for GC, approximately half of patients with gastritis are negative for *H. pylori* infection, and the abundance of *H. pylori* is decreasing in cancer patients. Lofgren JL et al. used a transgenic insulin-gastrin mouse model to confirm the association of gastric microbiota with the development of GC [109]. Sohn SH et al. used pyrosequencing to analyze the corpuscular microbiota and studied the possible roles of microbiota other than *H. pylori* in the development of GC [110]. Most studies on gastric microbes have shown that the most common non-*H. pylori* populations are thick-walled bacteria, *Bacteroides*, and *Fusobacterium* [111]. Hsieh YY et al. found that the number of *Clostridium* in gastric cancer patients in Taiwan is significantly higher than that in other gastrointestinal diseases [112]. Moreover, recent research indicated that the average age of patients with GC has been decreasing [113], which may be related to disorders of microbial metabolism in the stomach. These results also suggest that *H. pylori* is the primary microbe related to GC, while a variety of other microbes play secondary roles [114,115]. In addition to the above microbial factors, alcohol and tobacco intake are also related to the incidence of GC [116]. Although various experiments are currently underway to investigate whether the interaction of alcohol with *H. pylori* can trigger GC [117], these studies are complex, and so far, the relationship between *H. pylori* and alcohol does not appear to be significant. The high incidence of CagA-positive *H. pylori* infection and precancerous lesions may also be related to the use of tobacco. Smoking can cause stomach damage, impair the healing of peptic ulcers, increase *H. pylori* infection, and increase the recurrence rate of peptic ulcers [118]. Therefore, tobacco increases the incidence of GC. Although other microbiota and alcohol may not necessarily directly affect the occurrence of GC, they may amplify the effects of *H. pylori*, accelerating the development of GC. This is an exciting area, and will also be the direction of our next phase of research on GC.

***H. pylori* Induce Gastric Cancer in Patients with a Family History of GC**

Nishizawa T et al. performed single-factor and multivariate regression analyses that included the age, gender, body mass index, previous cancer history, family history of primary gastric cancer, and history of gastric ulcers in 206 patients with *H. pylori* infection. They concluded that family history is an independent risk factor for GC progression in patients with *H. pylori* infection [119]. At the same time, Sepulveda et al. found that patients with total gastritis with a family history of GC had higher lymphoid follicle density associated with *H. pylori* infection [120]. These two studies suggest that the interaction of *H. pylori* infection with a family history of GC increases the

incidence of GC in these individuals. This may be used as a theoretical basis for strategies to prevent GC. Because a family history of GC is associated with an increased risk of GC, the histological findings of gastritis may be suitable for the screening and monitoring of GC, especially in relatively young and at-risk populations. Infection with CagA-positive strains and records of family history may be useful markers for identifying patients at high risk for disease, as well as for prevention and early detection of GC.

Outlook

Microbiota is essential in the process of GC. Therefore, some measures against microbes (or that regulate specific populations of microbes) in the stomach may effectively reduce the incidence of GC. Leung WK et al. linked the treatment of *H. pylori* infection with lower risk of GC by analyzing data from Hong Kong public hospital databases [121]. Some laboratories attempted to inhibit IL-8 levels in the serum in a mouse model, and found that the pH of the stomach returned to normal levels, effectively inhibiting the viability of *H. pylori* [122]. Interestingly, some scholars have studied the anti-*H. pylori* effects of lactic acid bacteria (LAB). The concentration and morphology of organic acids were found to affect *H. pylori* infection [123]. In this way, doctors may be able to inhibit *H. pylori* activity by the delivery of new microbial populations. In addition, iron deficiency anemia [124] and high-salt [125] diets are factors that induce an increase in the invasiveness of *H. pylori*, so dietary interventions may be useful. However, the specific eradication of *H. pylori* seems to be the most promising approach to prevent GC. Human studies have confirmed that the eradication of *H. pylori* can reduce the risk of GC, a strategy that is more useful for patients without atrophic gastritis or intestinal metaplasia [126]. Of note, the presence of CagA-positive strains and a family history of GC provide useful markers for identifying patients at high risk for disease, which adds some accuracy to screening for gastric cancer. We anticipate that the technology used to both identify and eradicate *H. pylori* will be more targeted in the future, and the prevention and cure rates for GC will increase as a result of these and other interventions targeting the microbiome.

Abbreviations

Akt, protein kinase B;
C-Jun NH2-terminal kinase, JNK;
C-terminal src kinase, Csk;
Cyclo-oxygenase, COX-2;
Cytotoxin-associated gene A, CagA;
C10 regulator of kinase, Crk;
Extracellular regulated protein kinases, Erk;
Gastric cancer, GC;
Glu-Pro-Ile-Tyr-Ala, EPIYA;
Glutathione, GSH;
Growth factor receptor-bound 2, Grb2;

Helicobacter pylori, *H. Pylori*;
Heme oxygenase-1, HO-1;
Hepatocyte growth factor receptor, c-met;
Hydrogen peroxide, H₂O₂;
Lactic acid bacteria, LAB;
Mitogen-activated protein kinase, MAPK;
Mammalian target of rapamycin, m-TOR;
Myeloid differentiation factor 88, myd88;
NF-kappaB, NF-κB;
Partitioning-defective 1b, Par1b;
Phosphoinositide 3-kinase, PI3K;
Prostaglandin E2, PGE-2;
Reactive oxygen species, ROS;
Spermine oxidase, SMO;
Src family kinase, SFK;
Src-homology-2 domain containing ptpase, SHP2;
Toll-like receptor, TLR;
Transforming growth factor-β-activated kinase 1, TAK1;
Tumor necrosis factor receptor-associated factor 6, TRAF6;
Type IV secretion system, T4SS;
Vacuolating cytotoxin A, vacA

Conflicting Interests

The authors declare that there is no conflict of interests.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin* 2017;67(1):7-30.
2. Nie Y, Wu K, Yu J, Liang Q, Cai X, Shang Y, Zhou J, Pan K, Sun L, Fang J, Yuan Y, You W, Fan D. A global burden of gastric cancer: the major impact of China. *Expert Rev Gastroenterol Hepatol* 2017;11(7):651-61.
3. Shah MA. Gastric cancer: The gastric microbiota - bacterial diversity and implications. *Nat Rev Gastro Hepat* 2017;14(12):692-3.
4. Fock KM. Review article: the epidemiology and prevention of gastric cancer. *Aliment Pharm Therap* 2014;40(3):250-60.
5. Hirayama F, Takagi S, Kusuhara H, Iwao E, Yokoyama Y, Ikeda Y. Induction of gastric ulcer and intestinal metaplasia in mongolian gerbils infected with *Helicobacter pylori*. *J Gastroenterol* 1996;31(5):755-7.
6. Hirayama F, Takagi S, Yokoyama Y, Iwao E, Ikeda Y. Establishment of gastric *Helicobacter pylori* infection in Mongolian gerbils. *J Gastroenterol* 1996;31 Suppl 9: 24-28.
7. Wang LL, Yu XJ, Zhan SH, Jia SJ, Tian ZB, Dong QJ. Participation

of microbiota in the development of gastric cancer. *World J Gastroenterol* 2014;20(17):4948-52.

8. Gao JJ, Zhang Y, Gerhard M, Mejias-Luque R, Zhang L, Vieth M, Ma JL, Bajbouj M, Suchanek S, Liu WD, Ulm K, Quante M, Li ZX, Zhou T, Schmid R, Classen M, Li WQ, You WC, Pan KF. Association between gut microbiota and *Helicobacter pylori*-related gastric lesions in a high-risk population of gastric cancer. *Front Cell Infect Microbiol* 2018;8:202. doi: 10.3389/fcimb.

9. Cheung DY, Kim TH. *Helicobacter pylori* in human stomach: can it be called mutualism or a disease?. *Korean J Gastroenterol* 2012;59(5):329-37.

10. Ahmed N, Tenguria S, Nandanwar N. *Helicobacter pylori*--a seasoned pathogen by any other name. *Gut Pathog* 2009;1:24. doi: 10.1186/1757-4749-1-24.

11. Martinez LE, Hardcastle JM, Wang J, Pincus Z, Tsang J, Hoover TR, Bansil R, Salama NR. *Helicobacter pylori* strains vary cell shape and flagellum number to maintain robust motility in viscous environments. *Mol Microbiol* 2016;99(1):88-110.

12. Qin Z, Lin WT, Zhu S, Franco AT, Liu J. Imaging the motility and chemotaxis machineries in *Helicobacter pylori* by cryo-electron tomography. *J Bacteriol* 2017;199(3):e00695-16.

13. Kuwahara H, Miyamoto Y, Akaike T, Kubota T, Sawa T, Okamoto S, Maeda H. *Helicobacter pylori* urease suppresses bactericidal activity of peroxynitrite via carbon dioxide production. *Infect Immun* 2000;68(8):4378-83.

14. Aihara E, Closson C, Matthis AL, Schumacher MA, Engevik AC, Zavros Y, Ottemann KM, Montrose MH. Motility and chemotaxis mediate the preferential colonization of gastric injury sites by *Helicobacter pylori*. *PLoS pathogens* 2014;10(7): e1004275.

15. Khatoun J, Prasad KN, Prakash Rai R, Ghoshal UC, Krishnani N. Association of heterogeneity of *Helicobacter pylori* cag pathogenicity island with peptic ulcer diseases and gastric cancer. *Br J Biomed Sci* 2017;74(3):121-6.

16. Backert S, Schwarz T, Miehle S, Kirsch C, Sommer C, Kwok T, Gerhard M, Goebel UB, Lehn N, Koenig W, Meyer TF. Functional analysis of the cag pathogenicity island in *Helicobacter pylori* isolates from patients with gastritis, peptic ulcer, and gastric cancer. *Infect Immun* 2004;72(2):1043-56.

17. Tohidpour A, Gorrell RJ, Roujeinikova A, Kwok T. The middle fragment of *Helicobacter pylori* CagA induces actin rearrangement and triggers its own uptake into gastric epithelial cells. *Toxins (Basel)* 2017;9(8). doi: 10.3390/toxins8060187.

18. Martínez-Carrillo DN, Atrisco-Morales J, Hernández-Pando R, Reyes-Navarrete S, Betancourt-Linares R, Cruz-del Carmen I, Illades Aguiar B, Román-Román A, Fernández-Tilapa G. *Helicobacter pylori* vacA and cagA genotype diversity and interferon gamma expression in patients with chronic gastritis and patients with gastric cancer. *Rev Gastroenterol Mex* 2014;79(4):220-8.

19. Parsonnet J, Friedman GD, Orentreich N, Vogelmann H. Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. *Gut* 1997;40(3):297-301.

20. Azuma T, Ohtani M, Yamazaki Y, Higashi H, Hatakeyama M. Meta-analysis of the relationship between CagA seropositivity and gastric cancer. *Gastroenterology* 2004;126(7):1926-27; author reply 1927-8.

21. Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-anal-

ysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology* 2003;125(6):1636-44.

22. Sicinschi LA, Correa P, Peek RM, Camargo MC, Piazuelo MB, Romero-Gallo J, Hobbs SS, Krishna U, Delgado A, Mera R, Bravo LE, Schneider BG. CagA C-terminal variations in *Helicobacter pylori* strains from Colombian patients with gastric precancerous lesions. *Clin Microbiol Infect* 2010;16(4):369-78.

23. Zhang Y, Argent RH, Letley DP, Thomas RJ, Atherton JC. Tyrosine phosphorylation of CagA from Chinese *Helicobacter pylori* isolates in AGS gastric epithelial cells. *J Clin Microbiol* 2005;43(2):786-90.

24. Stein M, Bagnoli F, Halenbeck R, Rappuoli R, Fantl WJ, Covacci A. c-Src/Lyn kinases activate *Helicobacter pylori* CagA through tyrosine phosphorylation of the EPIYA motifs. *Mol Microbiol* 2002;43(4):971-80.

25. Krisch LM, Posselt G, Hammerl P, Wessler S. CagA Phosphorylation in *Helicobacter pylori*-Infected B Cells Is Mediated by the Non-receptor Tyrosine Kinases of the Src and Abl Families. *Infect Immun* 2016;84(9):2671-80.

26. Yong X, Tang B, Li BS, Xie R, Hu CJ, Luo G, Qin Y, Dong H, Yang SM. *Helicobacter pylori* virulence factor CagA promotes tumorigenesis of gastric cancer via multiple signaling pathways. *Cell Commun Signal* 2015;13:30. doi: 10.1186/s12964-015-0111-0.

27. Kocazeybek BS, Caliskan R, Erdamar Cetin S, Ergin S, Kuskucu M, Kepil N, Oyku Dinc H, Ziya Erzincan Y, Saribas S, Bahar Tokman H, Kalayci F, Akgul O, Yuksel P, Karakullukcu A, Ziver T, Sirekbasan S, Caglar E, Bal K. Patterns of EPIYA motifs among cagA-positive *Helicobacter pylori* strains: a case-control study in a Turkish population with Eurasian geographical features. *J Med Microbiol* 2015;64(10):1117-23.

28. Nagase L, Hayashi T, Senda T, Hatakeyama M. Dramatic increase in SHP2 binding activity of *Helicobacter pylori* Western CagA by EPIYA-C duplication: its implications in gastric carcinogenesis. *Sci Rep* 2015; 28;5:15749. doi: 10.1038/srep15749.

29. Liu X, Zheng H, Li X, Wang S, Meyerson HJ, Yang W, Neel BG, Qu CK. Gain-of-function mutations of Ptpn11 (Shp2) cause aberrant mitosis and increase susceptibility to DNA damage-induced malignancies. *Proc Natl Acad Sci U S A* 2016;113(4):984-9.

30. Hayashi T, Senda M, Suzuki N, Nishikawa H, Ben C, Tang C, Nagase L, Inoue K, Senda T, Hatakeyama M. Differential mechanisms for SHP2 binding and activation are exploited by geographically distinct *Helicobacter pylori* CagA oncoproteins. *Cell reports* 2017;20(12):2876-90.

31. Lee IO, Kim JH, Choi YJ, Pillinger MH, Kim SY, Blaser MJ, Lee YC. *Helicobacter pylori* CagA phosphorylation status determines the gp130-activated SHP2/ERK and JAK/STAT signal transduction pathways in gastric epithelial cells. *J Biol Chem* 2010;285(21):16042-50.

32. Hu ZQ, Ma R, Zhang CM, Li J, Li L, Hu ZT, Gao QI, Li WM. Expression and clinical significance of tyrosine phosphatase SHP2 in thyroid carcinoma. *Oncol Lett* 2015;10(3):1507-12.

33. Suzuki M, Mimuro H, Suzuki T, Park M, Yamamoto T, Sasakawa C. Interaction of CagA with Crk plays an important role in *Helicobacter pylori*-induced loss of gastric epithelial cell adhesion. *J Exp Med* 2005;202(9):1235-47.

34. Watanabe T, Tsuda M, Tanaka S, Ohba Y, Kawaguchi H, Majima T, Sawa H, Minami A. Adaptor protein Crk induces Src-dependent activation of p38 MAPK in regulation of synovial sarcoma cell proliferation.

Mol Cancer Res 2009;7(9):1582-92.

35. Tsutsumi R, Takahashi A, Azuma T, Higashi H, Hatakeyama M. Focal adhesion kinase is a substrate and downstream effector of SHP-2 complexed with *Helicobacter pylori* CagA. *Mol Cell Biol* 2006;26(1):261-76.
36. Chang CC, Kuo WS, Chen YC, Perng CL, Lin HJ, Ou YH. Fragmentation of CagA Reduces Hummingbird Phenotype Induction by *Helicobacter pylori*. *PLoS One* 2016;11(3):e0150061.
37. Zhu J. Csk/CD148 and platelet SFK activation: a balancing act! *Blood* 2018;131(10):1042-3.
38. Huang X, Wang C, Sun J, Luo J, You J, Liao L, Li M. Clinical value of CagA, c-Met, PI3K and Beclin-1 expressed in gastric cancer and their association with prognosis. *Oncol Lett* 2018;15(1):947-55.
39. Mimuro H, Suzuki T, Tanaka J, Asahi M, Haas R, Sasakawa C. Grb2 is a key mediator of *Helicobacter pylori* CagA protein activities. *Mol Cell* 2002;10(4):745-55.
40. Yamahashi Y, Hatakeyama M. PAR1b takes the stage in the morphogenetic and motogenetic activity of *Helicobacter pylori* CagA oncoprotein. *Cell Adh Migr* 2013;7(1):11-8.
41. Churin Y, Al-Ghoul L, Kepp O, Meyer TF, Birchmeier W, Naumann M. *Helicobacter pylori* CagA protein targets the c-Met receptor and enhances the motogenic response. *J Cell Biol* 2003;161(2):249-55.
42. Ye B, Jiang LL, Xu HT, Zhou DW, Li ZS. Expression of PI3K/AKT pathway in gastric cancer and its blockade suppresses tumor growth and metastasis. *Int J Immunopathol Pharmacol* 2012;25(3):627-36.
43. Murata-Kamiya N, Kurashima Y, Teishikata Y, Yamahashi Y, Saito Y, Higashi H, Aburatani H, Akiyama T, Peek RM, Jr., Azuma T, Hatakeyama M. *Helicobacter pylori* CagA interacts with E-cadherin and deregulates the beta-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. *Oncogene* 2007;26(32):4617-26.
44. Neal JT, Peterson TS, Kent ML, Guillemin K. H. *pylori* virulence factor CagA increases intestinal cell proliferation by Wnt pathway activation in a transgenic zebrafish model. *Dis Model Mech* 2013;6(3):802-10.
45. Yong X, Tang B, Xiao YF, Xie R, Qin Y, Luo G, Hu CJ, Dong H, Yang SM. *Helicobacter pylori* upregulates Nanog and Oct4 via Wnt/beta-catenin signaling pathway to promote cancer stem cell-like properties in human gastric cancer. *Cancer letters* 2016;374(2):292-303.
46. Cai C, Zhu X. The Wnt/beta-catenin pathway regulates self-renewal of cancer stem-like cells in human gastric cancer. *Mol Med Rep* 2012;5(5):1191-6.
47. Kim SY, Lee YC, Kim HK, Blaser MJ. *Helicobacter pylori* CagA transfection of gastric epithelial cells induces interleukin-8. *Cell Microbiol* 2006;8(1):97-106.
48. Chen Z, Liu M, Liu X, Huang S, Li L, Song B, Li H, Ren Q, Hu Z, Zhou Y, Qiao L. COX-2 regulates E-cadherin expression through the NF-kappaB/Snail signaling pathway in gastric cancer. *Int J Mol Med* 2013;32(1):93-100.
49. Snider JL, Allison C, Bellaire BH, Ferrero RL, Cardelli JA. The beta1 integrin activates JNK independent of CagA, and JNK activation is required for *Helicobacter pylori* CagA⁺-induced motility of gastric cancer cells. *J Biol Chem* 2008;283(20):13952-63.
50. Wandler AM, Guillemin K. Transgenic expression of the *Helicobacter pylori* virulence factor CagA promotes apoptosis or tumorigenesis through JNK activation in *Drosophila*. *PLoS pathogens* 2012;8(10):e1002939.
51. Rojanasakul Y. Linking JNK-STAT3-Akt signaling axis to EZH2 phosphorylation: a novel pathway of carcinogenesis. *Cell Cycle* 2013;12(2):202-3.
52. Lee KS, Kalantzis A, Jackson CB, O'Connor L, Murata-Kamiya N, Hatakeyama M, Judd LM, Giraud AS, Menheniott TR. *Helicobacter pylori* CagA triggers expression of the bactericidal lectin REG3gamma via gastric STAT3 activation. *PLoS One* 2012;7(2):e30786.
53. Lima VP, de Lima MA, Andre AR, Ferreira MV, Barros MA, Rabenhorst SH. H *pylori* (CagA) and Epstein-Barr virus infection in gastric carcinomas: correlation with p53 mutation and c-Myc, Bcl-2 and Bax expression. *World J Gastroentero* 2008;14(6):884-91.
54. Wei J, Noto JM, Zaika E, Romero-Gallo J, Piazuelo MB, Schneider B, El-Rifai W, Correa P, Peek RM, Zaika AI. Bacterial CagA protein induces degradation of p53 protein in a p14ARF-dependent manner. *Gut* 2015;64(7):1040-8.
55. Buti L, Spooner E, Van der Veen AG, Rappuoli R, Covacci A, Ploegh HL. *Helicobacter pylori* cytotoxin-associated gene A (CagA) subverts the apoptosis-stimulating protein of p53 (ASPP2) tumor suppressor pathway of the host. *Proc Natl Acad Sci U S A* 2011;108(22):9238-43.
56. Na YJ, Shim KN, Joo YH, Kim SE, Jung HK, Jung SA, Cho MS. RUNX3 methylation, loss of RUNX3 expression and clinicopathologic findings according to *Helicobacter pylori* CagA in gastric carcinoma. *Korean J Gastroentero* 2015;66(2):75-84.
57. Tsang YH, Lamb A, Romero-Gallo J, Huang B, Ito K, Peek RM, Jr., Ito Y, Chen LF. *Helicobacter pylori* CagA targets gastric tumor suppressor RUNX3 for proteasome-mediated degradation. *Oncogene* 2010;29(41):5643-50.
58. Ivie SE, McClain MS, Torres VJ, Algood HM, Lacy DB, Yang R, Blanke SR, Cover TL. *Helicobacter pylori* VacA subdomain required for intracellular toxin activity and assembly of functional oligomeric complexes. *Infect Immun* 2008;76(7):2843-51.
59. Torres VJ, Ivie SE, McClain MS, Cover TL. Functional properties of the p33 and p55 domains of the *Helicobacter pylori* vacuolating cytotoxin. *J Biol Chem* 2005;280(22):21107-14.
60. Torres VJ, McClain MS, Cover TL. Interactions between p-33 and p-55 domains of the *Helicobacter pylori* vacuolating cytotoxin (VacA). *J Biol Chem* 2004;279(3):2324-31.
61. Leunk RD, Johnson PT, David BC, Kraft WG, Morgan DR. Cytotoxic activity in broth-culture filtrates of *Campylobacter pylori*. *J Med Microbiol* 1988;26(2):93-9.
62. Tombola F, Carlesso C, Szabo I, de Bernard M, Reyat JM, Telford JL, Rappuoli R, Montecucco C, Papini E, Zoratti M. *Helicobacter pylori* vacuolating toxin forms anion-selective channels in planar lipid bilayers: possible implications for the mechanism of cellular vacuolation. *Biophys J* 1999;76(3):1401-9.
63. Czajkowsky DM, Iwamoto H, Cover TL, Shao Z. The vacuolating toxin from *Helicobacter pylori* forms hexameric pores in lipid bilayers at low pH. *Proc Natl Acad Sci U S A* 1999; 96(5):2001-6.
64. Ricci V, Sommi P, Fiocca R, Necchi V, Romano M, Solcia E. Extracellular pH modulates *Helicobacter pylori*-induced vacuolation and VacA toxin internalization in human gastric epithelial cells. *Biochem Biophys Res Commun* 2002;292(1):167-74.
65. Sun J, Wu Y, Su Z, Liu Z, Su B, Liu Z, Liu W, Zhao H, Tana, Ba-

- teer, Eshita Y, Chi B, Zhao L, Fang X, Hao W, Wu S, Bian J, Chen J, Ouyang X. The role of small molecular weight compounds to increase vacuolation induced by VacA toxin in vitro. *Toxicol In Vitro* 2010;24(5):1373-8.
66. Morbiato L, Tombola F, Campello S, Del Giudice G, Rappuoli R, Zoratti M, Papini E. Vacuolation induced by VacA toxin of *Helicobacter pylori* requires the intracellular accumulation of membrane permeant bases, Cl(-) and water. *FEBS Lett* 2001;508(3):479-83.
67. Ricci V. Relationship between VacA toxin and host cell autophagy in *Helicobacter pylori* infection of the human stomach: a few answers, many questions. *Toxins (Basel)* 2016;8(7).
68. Raju D, Jones NL. Methods to monitor autophagy in *H. pylori* vacuolating cytotoxin A (VacA)-treated cells. *Autophagy* 2010;6(1):138-43.
69. Jain P, Luo ZQ, Blanke SR. *Helicobacter pylori* vacuolating cytotoxin A (VacA) engages the mitochondrial fission machinery to induce host cell death. *Proc Natl Acad Sci U S A* 2011;108(38):16032-7.
70. Chatre L, Fernandes J, Michel V, Fiette L, Ave P, Arena G, Jain U, Haas R, Wang TC, Ricchetti M, Touati E. *Helicobacter pylori* targets mitochondrial import and components of mitochondrial DNA replication machinery through an alternative VacA-dependent and a VacA-independent mechanisms. *Scientific reports* 2017;7(1):15901.
71. Utsch C, Haas R. VacA's induction of VacA-Containing Vacuoles (VCVs) and their immunomodulatory activities on human t cells. *Toxins (Basel)* 2016;8(6).
72. Djekic A, Muller A. The Immunomodulator VacA promotes immune tolerance and persistent *Helicobacter pylori* infection through Its activities on T-cells and antigen-presenting cells. *Toxins (Basel)* 2016;8(6).
73. D'Elia MM, Montecucco C, de Bernard M. VacA and HP-NAP, Ying and Yang of *Helicobacter pylori*-associated gastric inflammation. *Clin Chim Acta* 2007;381(1):32-8.
74. Wang F, Xia P, Wu F, Wang D, Wang W, Ward T, Liu Y, Aikhionbare F, Guo Z, Powell M, Liu B, Bi F, Shaw A, Zhu Z, Elmoselhi A, Fan D, Cover TL, Ding X, Yao X. *Helicobacter pylori* VacA disrupts apical membrane-cytoskeletal interactions in gastric parietal cells. *J Biol Chem* 2008;283(39):26714-25.
75. Kobayashi H, Kamiya S, Suzuki T, Kohda K, Muramatsu S, Kurumada T, Ohta U, Miyazawa M, Kimura N, Mutoh N, Shirai T, Takagi A, Harasawa S, Tani N, Miwa T. The effect of *Helicobacter pylori* on gastric acid secretion by isolated parietal cells from a guinea pig. Association with production of vacuolating toxin by *H. pylori*. *Scand J Gastroenterol* 1996;31(5):428-33.
76. Supajatura V, Ushio H, Wada A, Yahiro K, Okumura K, Ogawa H, Hirayama T, Ra C. Cutting edge: VacA, a vacuolating cytotoxin of *Helicobacter pylori*, directly activates mast cells for migration and production of proinflammatory cytokines. *J Immunol* 2002;168(6):2603-7.
77. Fujikawa A, Shirasaka D, Yamamoto S, Ota H, Yahiro K, Fukada M, Shintani T, Wada A, Aoyama N, Hirayama T, Fukamachi H, Noda M. Mice deficient in protein tyrosine phosphatase receptor type Z are resistant to gastric ulcer induction by VacA of *Helicobacter pylori*. *Nat Genet* 2003;33(3):375-81.
78. Shirasaka D, Aoyama N, Sakashita M, Kuroda K, Maekawa S, Wambura CM, Miyamoto M, Tamura T, Yahiro K, Wada A, Kurazono H, Hirayama T, Kasuga M. Relationship between gastric ulcer and *Helicobacter pylori* VacA detected in gastric juice using bead-ELISA method. *Helicobacter* 2002;7(5): 281-6.
79. Bridge DR, Merrell DS. Polymorphism in the *Helicobacter pylori* CagA and VacA toxins and disease. *Gut microbes* 2013;4(2):101-17.
80. Tsugawa H, Suzuki H, Saya H, Hatakeyama M, Hirayama T, Hirata K, Nagano O, Matsuzaki J, Hibi T. Reactive oxygen species-induced autophagic degradation of *Helicobacter pylori* CagA is specifically suppressed in cancer stem-like cells. *Cell Host Microbe* 2012;12(6):764-77.
81. Tsugawa H. [Study of infection strategies of *Helicobacter pylori* and host cell response against CagA oncoprotein]. *Nihon Saikingaku Zasshi* 2014;69(4):565-75.
82. Montealegre OM, Jaramillo HC, Montealegre LG, Parra GG, Echeverry de PM, Delgado PMP. [Molecular and histological detection of *Helicobacter pylori* and genotyping based on babA2 and iceA in patients with benign gastric pathologies]. *Rev Chilena Infectol* 2010;27(2):112-8.
83. Chomvarin C, Namwat W, Chaicumpar K, Mairiang P, Sangchan A, Sripa B, Tor-Udom S, Vilaichone RK. Prevalence of *Helicobacter pylori* vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients. *Int J Infect Dis* 2008;12(1):30-6.
84. Podzorski RP, Podzorski DS, Wuerth A, Tolia V. Analysis of the vacA, cagA, cagE, iceA, and babA2 genes in *Helicobacter pylori* from sixty-one pediatric patients from the Midwestern United States. *Diagn Microbiol Infect Dis* 2003;46(2):83-8.
85. Gatti LL, Modena JL, Payao SL, Smith Mde A, Fukuhara Y, Modena JL, de Oliveira RB, Brocchi M. Prevalence of *Helicobacter pylori* cagA, iceA and babA2 alleles in Brazilian patients with upper gastrointestinal diseases. *Acta Trop* 2006;100(3):232-40.
86. Szczepanik M. Interplay between *Helicobacter pylori* and the immune system. Clinical implications. *J Physiol Pharmacol* 2006;57 Suppl 3:15-27.
87. Lee SK, Josenhans C. *Helicobacter pylori* and the innate immune system. *Int J Med Microbiol* 2005;295(5):325-34.
88. Sharma SA, Tummuru MK, Blaser MJ, Kerr LD. Activation of IL-8 gene expression by *Helicobacter pylori* is regulated by transcription factor nuclear factor-kappa B in gastric epithelial cells. *J Immunol* 1998;160(5):2401-7.
89. Kim Y, Seo JH, Kim H. beta-Carotene and lutein inhibit hydrogen peroxide-induced activation of NF-kappaB and IL-8 expression in gastric epithelial AGS cells. *J Nutr Sci Vitaminol (Tokyo)* 2011;57(3):216-23.
90. Zou J, Shankar N. Roles of TLR/MyD88/MAPK/NF-kappaB signaling pathways in the regulation of phagocytosis and proinflammatory cytokine expression in response to *E. faecalis* infection. *PLoS One* 2015;10(8): e0136947.
91. Lamb A, Yang XD, Tsang YH, Li JD, Higashi H, Hatakeyama M, Peek RM, Blanke SR, Chen LF. *Helicobacter pylori* CagA activates NF-kappaB by targeting TAK1 for TRAF6-mediated Lys 63 ubiquitination. *EMBO Rep* 2009;10(11):1242-9.
92. Chao X, Zao J, Xiao YG, Li JM, Tao S. Blocking of PI3K/AKT induces apoptosis by its effect on NF-kappaB activity in gastric carcinoma cell line SGC7901. *Biomed Pharmacother* 2010;64(9):600-4.
93. Jabbour HN, Milne SA, Williams AR, Anderson RA, Boddy SC. Expression of COX-2 and PGE synthase and synthesis of PGE(2) in

endometrial adenocarcinoma: a possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors. *Brit J Cancer* 2001;85(7):1023-31.

94. Tang M, Wang Y, Han S, Guo S, Xu N, Guo J. Endogenous PGE(2) induces MCP-1 expression via EP4/p38 MAPK signaling in melanoma. *Oncol Lett* 2013;5(2):645-50.

95. Chaturvedi R, de Sablet T, Peek RM, Wilson KT. Spermine oxidase, a polyamine catabolic enzyme that links *Helicobacter pylori* CagA and gastric cancer risk. *Gut microbes* 2012;3(1):48-56.

96. Chaturvedi R, Asim M, Romero-Gallo J, Barry DP, Hoge S, de Sablet T, Delgado AG, Wroblewski LE, Piazzuelo MB, Yan F, Israel DA, Casero RA, Jr., Correa P, Gobert AP, Polk DB, Peek RM, Jr., Wilson KT. Spermine oxidase mediates the gastric cancer risk associated with *Helicobacter pylori* CagA. *Gastroenterology* 2011;141(5):1696-708 e1691-2.

97. Arancia G, Calcabrini A, Marra M, Crateri P, Artico M, Martone A, Martelli F, Agostinelli E. Mitochondrial alterations induced by serum amine oxidase and spermine on human multidrug resistant tumor cells. *Amino Acids* 2004;26(3):273-82.

98. Bagchi D, Bhattacharya G, Stohs SJ. Production of reactive oxygen species by gastric cells in association with *Helicobacter pylori*. *Free Radic Res* 1996;24(6):439-50.

99. Piao JY, Lee HG, Kim SJ, Kim DH, Han HJ, Ngo HK, Park SA, Woo JH, Lee JS, Na HK, Cha YN, Surh YJ. *Helicobacter pylori* Activates IL-6-STAT3 signaling in human gastric cancer cells: potential roles for reactive oxygen species. *Helicobacter* 2016;21(5):405-16.

100. Zhou Y, Xia L, Liu Q, Wang H, Lin J, Oyang L, Chen X, Luo X, Tan S, Tian Y, Su M, Wang Y, Chen P, Wu Y, Wang H, Liao Q. Induction of pro-inflammatory response via activated macrophage-mediated NF-kappaB and STAT3 pathways in gastric cancer cells. *Cell Physiol Biochem* 2018;47(4):1399-410.

101. Hryciak EG, Bandiera SM. Involvement of cytochrome P450 in reactive oxygen species formation and cancer. *Adv Pharmacol* 2015;74:35-84.

102. Gobert AP, Verriere T, Asim M, Barry DP, Piazzuelo MB, de Sablet T, Delgado AG, Bravo LE, Correa P, Peek RM, Jr., Chaturvedi R, Wilson KT. Heme oxygenase-1 dysregulates macrophage polarization and the immune response to *Helicobacter pylori*. *J Immunol* 2014;193(6):3013-22.

103. Gobert AP, Asim M, Piazzuelo MB, Verriere T, Scull BP, de Sablet T, Glumac A, Lewis ND, Correa P, Peek RM, Jr., Chaturvedi R, Wilson KT. Disruption of nitric oxide signaling by *Helicobacter pylori* results in enhanced inflammation by inhibition of heme oxygenase-1. *J Immunol* 2011;187(10):5370-9.

104. Li N, Tang B, Jia YP, Zhu P, Zhuang Y, Fang Y, Li Q, Wang K, Zhang WJ, Guo G, Wang TJ, Feng YJ, Qiao B, Mao XH, Zou QM. *Helicobacter pylori* CagA protein negatively regulates autophagy and promotes inflammatory response via c-Met-PI3K/Akt-mTOR signaling pathway. *Front Cell Infect Mi* 2017;7:417.

105. Hisatsune J, Nakayama M, Isomoto H, Kurazono H, Mukaida N, Mukhopadhyay AK, Azuma T, Yamaoka Y, Sap J, Yamasaki E, Yahiro K, Moss J, Hirayama T. Molecular characterization of *Helicobacter pylori* VacA induction of IL-8 in U937 cells reveals a prominent role for p38MAPK in activating transcription factor-2, cAMP response element binding protein, and NF-kappaB activation. *J Immunol*

2008;180(7):5017-27.

106. Iwamoto H, Czajkowsky DM, Cover TL, Szabo G, Shao Z. VacA from *Helicobacter pylori*: a hexameric chloride channel. *FEBS Lett* 1999;450(1-2):101-4.

107. Luo JJ, Li CY, Liu S, Yu W, Tang SY, Cai HL, Zhang Y. Overexpression of *Helicobacter pylori* VacA N-terminal fragment induces proinflammatory cytokine expression and apoptosis in human monocytic cell line through activation of NF-kappaB. *Can J Microbiol* 2013;59(8):523-33.

108. Tongtawee T, Wattanawongdon W, Simawaranon T, Kaewpitoon S, Kaengpenkae S, Jintabanditwong N, Tangjanyatham P, Ratchapol W, Kangwantis K, Dechsukhum C, Leeanansaksiri W, Kaewpitoon N, Matrakool L, Panpimanmas S. Expression of Cancer Stem Cell Marker CD44 and Its Polymorphisms in Patients with Chronic Gastritis, Precancerous Gastric Lesion, and Gastric Cancer: A Cross-Sectional Multicenter Study in Thailand. *BioMed research international* 2017;2017:4384823.

109. Lofgren JL, Whary MT, Ge Z, Muthupalani S, Taylor NS, Mobley M, Potter A, Varro A, Eibach D, Suerbaum S, Wang TC, Fox JG. Lack of commensal flora in *Helicobacter pylori*-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. *Gastroenterology* 2011;140(1):210-20.

110. Sohn SH, Kim N, Jo HJ, Kim J, Park JH, Nam RH, Seok YJ, Kim YR, Lee DH. Analysis of gastric body microbiota by pyrosequencing: possible role of bacteria other than *Helicobacter pylori* in the gastric carcinogenesis. *J Cancer Prev* 2017;22(2):115-25.

111. Li J, Perez Perez GI. Is There a role for the non-helicobacter pylori bacteria in the risk of developing gastric cancer? *Int J Mol Sci* 2018;19(5).

112. Hsieh YY, Tung SY, Pan HY, Yen CW, Xu HW, Lin YJ, Deng YF, Hsu WT, Wu CS, Li C. Increased abundance of clostridium and fusobacterium in gastric microbiota of patients with gastric cancer in taiwan. *Sci Rep* 2018;8(1):158.

113. Zhou F, Shi J, Fang C, Zou X, Huang Q. Gastric carcinomas in young (Younger than 40 Years) chinese patients: clinicopathology, family history, and postresection survival. *Medicine (Baltimore)* 2016;95(9):e2873.

114. Jo HJ, Kim J, Kim N, Park JH, Nam RH, Seok YJ, Kim YR, Kim JS, Kim JM, Kim JM, Lee DH, Jung HC. Analysis of gastric microbiota by pyrosequencing: minor role of bacteria other than *Helicobacter pylori* in the gastric carcinogenesis. *Helicobacter* 2016;21(5):364-74.

115. De Witte C, Schulz C, Smet A, Malfertheiner P, Haesebrouck F. Other *Helicobacters* and gastric microbiota. *Helicobacter* 2016;21 Suppl 1:62-8.

116. Dong J, Thrift AP. Alcohol, smoking and risk of oesophago-gastric cancer. *Best Pract Res Clin Gastroenterol* 2017;31(5):509-17.

117. Ma SH, Jung W, Weiderpass E, Jang J, Hwang Y, Ahn C, Ko KP, Chang SH, Shin HR, Yoo KY, Park SK. Impact of alcohol drinking on gastric cancer development according to *Helicobacter pylori* infection status. *Br J Cancer* 2015;113(9):1381-8.

118. Siman JH, Forsgren A, Berglund G, Floren CH. Tobacco smoking increases the risk for gastric adenocarcinoma among *Helicobacter pylori*-infected individuals. *Scand J Gastroenterol* 2001; 36(2):208-13.

119. Nishizawa T, Suzuki H, Sakitani K, Yamashita H, Yoshida S, Hata K, Kanazawa T, Fujiwara N, Kanai T, Yahagi N, Toyoshima O. Family

history is an independent risk factor for the progression of gastric atrophy among patients with *Helicobacter pylori* infection. *United European gastroenterol J* 2017;5(1):32-6.

120. Sepulveda A, Peterson LE, Shelton J, Gutierrez O, Graham DY. Histological patterns of gastritis in *H. pylori*-infected individuals with a family history of gastric cancer. *Am J Gastroenterol* 2002;97(6):1365-70.

121. Leung WK, Wong IOL, Cheung KS, Yeung KF, Chan EW, Wong AYS, Chen L, Wong ICK, Graham DY. Effects of *Helicobacter pylori* treatment on incidence of gastric cancer in older individuals. *Gastroenterology*. 2018;155(1):67-75.

122. Kim JE, Kim MS, Yoon YS, Chung MJ, Yum DY. Use of selected lactic acid bacteria in the eradication of *Helicobacter pylori* infection. *J Microbiol* 2014;52(11):955-62.

123. Lin WH, Lin CK, Sheu SJ, Hwang CF, Ye WT, Hwang WZ, Tsen HY. Antagonistic activity of spent culture supernatants of lactic acid bacteria against *Helicobacter pylori* growth and infection in human gastric epithelial AGS cells. *J Food Sci* 2009;74(6):M225-30.

124. Burns M, Amaya A, Bodi C, Ge Z, Bakthavatchalu V, Ennis K, Wang TC, Georgieff M, Fox JG. *Helicobacter pylori* infection and low dietary iron alter behavior, induce iron deficiency anemia, and modulate hippocampal gene expression in female C57BL/6 mice. *PLoS One* 2017;12(3):e0173108.

125. Loh JT, Gaddy JA, Algood HM, Gaudieri S, Mallal S, Cover TL. *Helicobacter pylori* adaptation in vivo in response to a high-salt diet. *Infect Immun* 2015;83(12):4871-83.

126. Cheung TK, Wong BC. Treatment of *Helicobacter pylori* and prevention of gastric cancer. *J Dig Dis* 2008;9(1):8-13.

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