Review

Helicobacter pylori infection and oxidative stress

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***Helicobacter pylori* (*H. pylori*) infection promotes the migration of polymorphonuclear leukocytes from the gastric mucosal microcirculation through chemokine induction, leading to the excessive production of ROS. Like eukaryotes, *H. pylori* possesses superoxide dismutase and catalase, and is resistant to ROS from host polymorphonuclear leukocytes. Oxidants such as monochloramine produced by ROS cause chronic inflammation in the gastric mucosa. *H. pylori*-derived virulence factor m1-type VacA induces intracellular ROS accumulation and autophagy, which degrades the *H. pylori*-derived oncoprotein, CagA. In CD44v9-positive gastric cancer stem-like cells, reduced-type glutathione levels increase within the cell because of the cystine transporter on the cell surface, wherein oxidative stress-induced autophagy no longer occurs. As a result, the oncoprotein CagA accumulates in the cells, thus becoming tumorigenic.**

***Key Words*: ROS, CagA, VacA, cancer-stem cell, CD44v9**

Helicobacter pylori (*H. pylori*) was first isolated and cultured from the gastric mucosa of patients with duodenal ulcers by Marshall and Warren.[1] *H. pylori* is a Gram-negative spiral rod, with a diameter of approximately 0.5 μm and a length of approximately 3.5 μm.[2] It is now widely recognized as an important pathogenic factor not only in gastric/duodenal ulcers and chronic gastritis, but also in gastric MALT lymphoma, gastric cancer, and immune thrombocytopenic purpura (ITP).[3] Infection with *H. pylori* often occurs mainly during childhood. Although the initial infection only causes acute gastritis and diarrhea, once established, it persists throughout a lifetime. One oxidant specific to *H. pylori* infection is monochloramine. Monochloramine causes gastric mucosal cell damage; it is produced when ROS and hypochlorous acid radicals, generated by infiltrating neutrophils, react with ammonia. Ammonia is derived from urease produced by *H. pylori*.[4] Furthermore, the vacuolating cytotoxin, VacA,[5] produced by *H. pylori* induces chronic gastric mucosal inflammation. This review provides an overview of the various types of oxidative stress that occur during *H. pylori* infection.

Host Gastric Mucosal Response to *H. pylori*

H. pylori adheres to gastric surface mucus cells (gastric pit epithelium) or forms microcolonies in the mucus gel layer derived from these surface epithelial cells. In an *H. pylori*-infected host gastric mucosa, the extravasation of polymorphonuclear leukocytes from the gastric mucosal microcirculation is promoted through the induction of chemokines, where ROS are released into the mucosa.

Originally, excessive ROS production by neutrophils was considered an elimination response to *H. pylori*; however, *H. pylori*, like eukaryotes, possesses superoxide dismutase (SOD) and catalase (Kat), thus exerting resistance. As a result, excessive ROS production persists in *H. pylori*-infected gastric mucosa, wherein gastric mucosal damage occurs due to ROS accumulation, including superoxide (O_2^-) and hydrogen peroxide (H_2O_2). In *H. pylori*-infected gastric mucosa, myeloperoxidase (MPO), which is released through the degranulation of polymorphonuclear leukocytes, produces hypochlorous anions (OCl^-) from H_2O_2 in the presence of Cl^-. OCl^- reacts with the ammonia produced by *H. pylori* urease, using urea as a substrate, to produce monochloramine (NH_2Cl). NH_2Cl is highly toxic, fat-soluble, and easily passes through cell membranes. It then induces significant epithelial cell destruction in *H. pylori*-infected mucosa. This mechanism of epithelial cell damage, as mediated by NH_2Cl production and specific to *H. pylori*-infected gastric mucosa, is thought to be an important contributing factor in the induction of gastric mucosal disease caused by *H. pylori*.[6–8]

Antioxidant System in *H. pylori*

H. pylori is a microaerophilic bacterium; in an environment with low oxygen concentrations, oxygen becomes the final acceptor of electrons during the energy metabolism process, which is similar to aerobic bacteria and thus results in the generation of active oxygen ($O_2 + e^- \rightarrow O_2^{\bullet-}$). Furthermore, because *H. pylori* infects the gastric mucosa of the host, it is constantly exposed to ROS stimulation as a result of foreign body clearance. *H. pylori* must prevent ROS exposure through numerous mechanisms to maintain vital functions; therefore, they produce antioxidants that neutralize ROS. SOD is an enzyme that catalyzes the redox reaction ($O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow O_2 + H_2O_2$), thus disproportioning $O_2^{\bullet-}$ into oxygen and H_2O_2.

SOD is classified into three types, depending on the metal ion at their center: (1) copper-zinc superoxide dismutase (Cu, Zn-SOD; bound to both Cu and Zn), (2) iron/manganese superoxide dismutase (Fe/Mn-SOD; bound to either Fe or Mn), and (3) nickel superoxide dismutase (Ni-SOD; bound to Ni). *E. coli* expresses Cu-and Zn-SOD in the periplasm, and Fe-SOD and Mn-SOD in the cytoplasm.[9]

In contrast, *H. pylori* possesses only one type of Fe-SOD. The mechanism by which *H. pylori* Fe-SOD (Hp-SOD) exerts ROS resistance in the host gastric mucosa has not been elucidated. The C-terminal structure of Hp-SOD is longer than that of Fe-SOD in other bacteria by approximately 20 amino acid residues. This structural change may explain why SOD alone exhibited sufficient ROS resistance.[10] Hp-SOD is tightly regulated at the transcriptional level by the ferric uptake regulator (Fur).[7] The FecA proteins (FecA1, FecA2, and FecA3), a family of Fe ion (Fe^{3+}-dicitrate) transporters, are present in the outer membrane of *H. pylori*. Hp-SOD activity is significantly reduced, particularly in FecA1-deficient *H. pylori*. This suggests that the Fe ions

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necessary to maintain Hp-SOD activity are supplied by FecA1.[11] In addition, Hp-SOD transcription is suppressed by the binding of non-ferrous, ion-binding Fur (apo-Fur) to the promoter region (Fur-box) located upstream of the Hp-SOD gene (−5 to −47 region). However, upon receiving an iron supply, iron ion-bound Fur dissociates from the Fur-box, thereby resulting in enhanced transcription of Hp-SOD.

Metronidazole (MET), an antibacterial drug, is the mainstay of secondary eradication therapy, particularly in Japan. Metronidazole is a prodrug; although devoid of antibacterial activity, it can diffuse through membranes and be transported into the bacterial body, where it becomes active only after being reduced.[12,13] We discovered that MET resistance is caused not only by mutations in RdxA, as previously reported,[11] but also by Fur, a regulatory factor for iron intake. In particular, two amino acid mutations in Fur are important.[14] As mentioned above, the iron transporter FecA1 on the bacterial cell membrane determines the oxidative stress resistance of the bacterium.[11] Therefore, bacterial eradication treatment using FecA1-targeting compounds may be a potential alternative to conventional antibacterial therapy.[15]

H. pylori: Pathogenic Factors and Oxidative Stress

H. pylori produces several virulence factors, including the cag pathogenicity island (cagPAI), a set of genes encoding the type IV secretion apparatus and its effector molecule CagA, which is a particularly important virulence gene group. The retention rate of cagPAI was approximately 60% for the Western strains and over 90% for the East Asian strains. The type IV secretion apparatus effector protein, CagA, is a large protein consisting of approximately 1,200 amino acids, with a molecular weight of approximately 130,000. CagA-positive *H. pylori* infected individuals had a significantly higher incidence of gastric cancer than CagA-negative *H. pylori*-infected individuals. CagA was inserted into the gastric mucosal cells via a type IV secretion apparatus. Once inside the cell, CagA interacts with various intracellular target proteins, including the oncoprotein SHP2 and cell polarity protein PAR1, through the C-terminal EPIYA and CM motifs, thereby leading to the generation of abnormal cell proliferation signals. CagA is known to disrupt the polarity of epithelial cells and induce apoptosis in immune cells, such as lymphocytes and macrophages, in a mitochondrial pathway-dependent manner,[13] thus functioning as an onco-protein.[16] Therefore, CagA transfer into cells is thought to contribute to tumorigenicity; gastric tumors have been shown to develop in CagA-transgenic mice.[17]

CagA may not be eliminated from host gastric mucosal cells, despite being a foreign protein. Therefore, we investigated whether CagA exists stably in host cells. We infected AGS gastric epithelial cells with *H. pylori in-vitro* and examined their stability. Internalized CagA is degraded via autophagy and disappears within 24 h. In WT-A10 cells expressing CagA, autophagy of CagA did not occur, thereby suggesting that *H. pylori* bacterial factors other than CagA were necessary to trigger autophagy. Additionally, we found that the toxin VacA, produced by *H. pylori*, binds to the cell surface LRP1 protein to induce CagA-degrading autophagy.[18,19] The structure of VacA consists of an N-terminal fragment (p33 fragment), which is important for host vacuole formation, and a p55 fragment, which plays a role in receptor recognition. The p55 fragment contains a mid-region, which is important for binding to target cells; due to primary structural differences in this region, m1-type VacA (m1VacA) and m2-type VacA (m2VacA) can be broadly classified. Unlike m2VacA, m1VacA induces the accumulation of cellular ROS. Furthermore, reduced-type glutathione, which has an antioxidant effect, is decreased in cells due to ROS production by m1VacA. In other words, autophagy-induced oxidative stress is regulated by changes in the balance between pro-oxidants and antioxidants within cells. Intracellular ROS accumulation induced by m1VacA activates the Akt-Mdm2-p53 pathway and induces autophagy.[11] In the normal gastric mucosal epithelium, *H. pylori*-derived CagA is degraded by oxidative stress-induced autophagy and therefore cannot exert tumorigenicity (Fig. 1). In contrast, cancer stem cell precursors appear in the gastric mucosa and become precancerous lesions due to inflammatory changes. Cells expressing CD44v9, a splice variant of CD44 (CD44v9, a marker for gastric cancer stem-like cells; cancer stems that are thought to exist within CD44v9-positive cell clusters), have attracted attention. Cystine transporter (xCT) is stabilized on CD44v9-positive cell membranes and can take up large amounts of cystine. In these cells, excess glutathione can also be stored using cystine; therefore, its strong scavenging action prevents ROS accumulation.[12] When CD44v9-expressing cells were infected with *H. pylori*, ROS accumulation caused by m1VacA did not occur because of the abundance of intracellular glutathione (Fig. 2). Thus, CagA-mediated autophagy was not activated. Consequently, intracellular CagA is stabilized without degradation, thereby increasing the risk for gastric cancer development. In the endoscopic therapy of early gastric cancer, the risk for metachronous gastric cancer was significantly increased in cancers that exhibited high CD44v9 expression, as compared to those with low expression.[20] We previously reported that cells overexpressing the actin polymerization regulatory protein, CAPZA1 (capping the actin protein of muscle Z-line alpha subunit 1), became progenitor cells of CD44v9-positive cancer stem cells in human gastric cancer tissues. Furthermore, we showed that functional regulation of CAPZA1 leads to the inhibition of CD44v9-positive gastric cancer stem cell development.[21,22] As chronic *H. pylori* infection progresses to precancerous changes, such as chronic persistent gastritis, chronic atrophic gastritis, and intestinal metaplasia,[23] CAPZA1-positive cells are generated, into which CagA is injected; CD44v9-positive gastric cancer stem cells are then induced. CagA is no longer degraded or eliminated by autophagy, thereby triggering tumorigenic cell signalling.

Recent Progress of the Research Related to *H. pylori* Infection and Oxidative Stress

Gong *et al.*[24] employed liquid chromatography-mass spectrometry to determine serum proteome signatures and related pathways in individuals with gastric precancerous (pre-gastric cancer; pre-GC) lesions and gastric cancer (GC) and explored the effect of *H. pylori* infection. They identified differentially expressed proteins in the GC and pre-GC groups as compared with the non-atrophic gastritis (NAG) group. APOA4, a protein associated with metaplastic differentiation, and COMP, an extracellular matrix protein, were increased in the serum of patients with pre-GC and GC. In addition, several inflammation-associated proteins, such as component C3, were decreased in the GC and pre-GC groups, thus highlighting the tendency for the inflammatory response to converge at the gastric lesion during the GC-development cascade. Moreover, the abundance of proteins associated with radical scavenging was higher in the GC group than in the NAG group; these proteins were also increased in the serum of the *H. pylori*-positive GC group as compared to the *H. pylori*-negative GC group, thus reflecting the importance of oxidative stress pathways in *H. pylori* infection.

Uncontrolled immune responses due to *H. pylori* infection further produce excess reactive oxygen and nitrogen species (ROS and RNS), which lead to mucosal damage. Persistent oxidative stress is a major cause of gastric cancer. *H. pylori* regulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs), nitric oxide synthase 2 (NOS2), and polyamines to control the release of ROS and RNS via lesser-known mechanisms. The ROS and RNS produced by these pathways differentiate the macrophages and T cells from protective to inflammatory

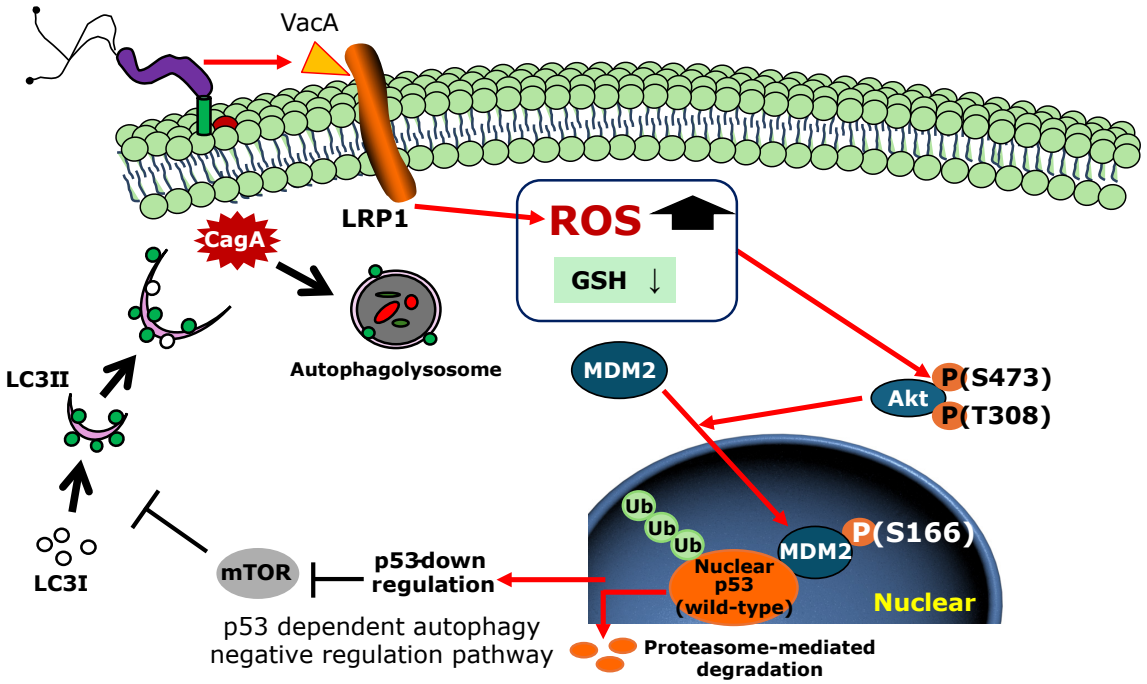

Fig. 1. CagA-degrading autophagy pathway in gastric epithelial cells. In the normal gastric mucosal epithelium, intracellular ROS accumulation by m1VacA activates the Akt-Mdm2-p53 pathway and induces autophagy. Then, *H. pylori*-derived CagA is degraded by oxidative stress-induced autophagy, and therefore cannot exert its tumorigenicity in these cells.

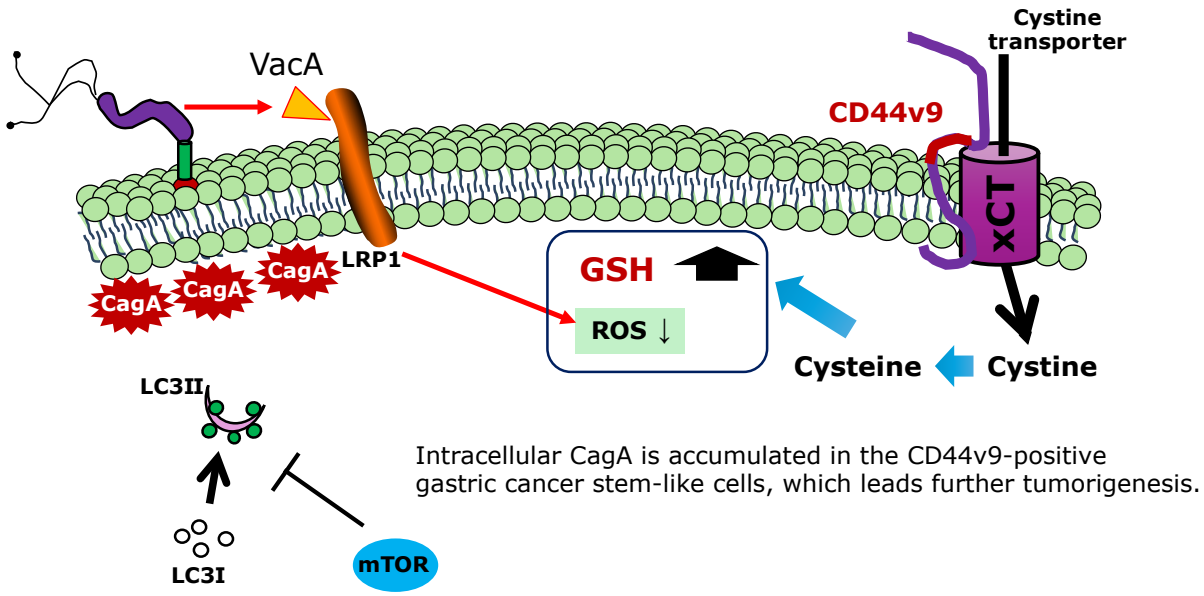

Fig. 2. Attenuated CagA-degrading autophagy pathway in gastric CD44v9-expressing epithelial cells. On CD44v9-positive cell membranes, the cystine transporter (xCT) is stabilized; these cells can take up large amounts of cystine and store glutathione in excess. Therefore, its strong scavenging action stops ROS accumulation. When *H. pylori* infects CD44v9-expressing cells, ROS accumulation caused by m1VacA does not occur due to the abundance of intracellular glutathione; thus, CagA-degrading autophagy is not activated.

phenotypes. Pathogen-associated molecular pattern (PAMPs)-induced ROS activate the nuclear oligomerization domain (NOD), leucine-rich repeats (LRR), and pyrin domain-containing protein 3 (NLRP3) inflammasomes for the release of pro-inflammatory cytokines.

On the other hand, Kumar and Dhiman evaluated the role of *H. pylori*-secreted concentrated proteins (HPSCP) and their relationship with oxidative stress, with regards to NLRP3 inflammasome activation and macrophage differentiation. They concluded that multiple pathways generate ROS during *H. pylori* infection, which further regulate other cellular oxidative processes. NO is closely associated with MPO and NLRP3 inflammasome inhibi-

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tion. Low levels of NO and MPO regulate gastrointestinal tract homeostasis and overcome the inflammatory response induced by NLRP3. ROS also play a crucial role in macrophage polarization and hence alter immune responses during *H. pylori* pathogenesis.[25]

Noszka *et al.*[26] presented a comprehensive study of the redox switch protein, HP1021, by combining transcriptomic, proteomic, and DNA-protein interaction analyses. Their results indicated that HP1021 modulates *H. pylori* response to oxidative stress. HP1021 controls typical ROS response pathways (katA and rocF) as well as a few canonical pathways, particularly DNA uptake and central carbohydrate metabolism. HP1021 is a molecular regulator of competence in *H. pylori*, as HP1021-dependent repression of comB DNA uptake genes is relieved under oxidative conditions, thereby increasing natural competence. Finally, they stated that HP1021 controls glucose consumption by directly regulating the gluP transporter and has an important impact on maintaining cellular energy balance.[26]

Urolithin B (UB) is another major intestinal metabolite of ellagic acid (EA) that possesses anti-inflammatory, antioxidant, and anti-apoptotic biological activities. Yu *et al.*[27] explored the effects of UB on *H. pylori* infection. They showed that UB reduced the adhesion and colonization of *H. pylori* as well as improved *H. pylori*-induced inflammation and oxidative stress. Moreover, UB had better anti-inflammatory and antioxidant effects than clarithromycin (CLR) and metronidazole (MET). In addition to inhibiting the secretion of CagA, UB reduced tissue damage caused by *H. pylori* infection. Taken together, intestinal UB was effective in improving oxidative damage caused by *H. pylori*.

Future Outlook

As a part of the inflammatory foreign body clearance response, infection with *H. pylori* causes excessive ROS production in the host gastric mucosa. *H. pylori* exhibits ROS resistance and is capable of persistent infection, thus causing a sustained inflammatory response and inducing chronic gastric mucosal epithelial cell damage via monochloramine and other agents. In contrast, ROS production is important for autophagy, a cellular response that eliminates bacteria-derived oncoproteins. CD44v9-positive cancer stem cells with acquired ROS resistance are found in the gastric mucosa and develop precancerous changes, thus resulting in tumorigenicity.

In any case, once *H. pylori* infection is confirmed, eradication therapy should be administered as early as possible before precancerous changes occur. In Japan, it has been 11 years (February 2013) since *H. pylori* eradication therapy for "*Helicobacter pylori*-infected gastritis" became covered by national insurance. Several eradication treatments have been developed in the past decade; the recent appearance of vonoprazan as a PCAB has significantly improved the eradication rate;[28] however, *H. pylori* infection has not been completely eradicated in Japan. Even if *H. pylori* infection is completely eradicated, other pathogens including microbiota may still be present.

Currently, treatment up to secondary eradication, which is covered by insurance, is expected to achieve an eradication success rate of >95%. However, the existence of cases that do not respond to eradication therapy as well as increasing drug-resistant bacteria pose a challenge. In successful tertiary and succeeding treatment lines for *H. pylori*, there is an urgent need to develop a new eradication paradigm that targets the antioxidant system of *H. pylori*.

Here, we describe the pathogenesis of oxidative stress and inflammation in the stomach caused by a single bacterium, *H. pylori*. It was thought that this occurs between Helicobacter species other than *H. pylori* or between individual bacteria other than *H. pylori* and the host gastric mucosa. In the future, it may be necessary to consider gastric diseases from larger data analyses, accounting for the totality of gastric microorganisms, environmental factors, and multi-omics of host gastric mucosa.

Conflict of Interest

The author (HS) received research funds from Tohso Co. and Biofermin Pharmaceutical Co., Ltd., and received service honoraria from Otsuka Pharmaceutical Co. and Takeda Pharmaceutical Co. The author (MH) has declared no conflicts of interest.

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