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Molecular mechanism of *Helicobacter pylori*-induced autophagy in gastric cancer (Review)

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Abstract. *Helicobacter pylori* (*H. pylori*) is a gram-negative pathogen that colonizes gastric epithelial cells. The drug resistance rates of *H. pylori* have dramatically increased, causing persistent infections. Chronic infection by *H. pylori* is a critical cause of gastritis, peptic ulcers and even gastric cancer. In host cells, autophagy is stimulated to maintain cellular homeostasis following intracellular pathogen recognition by the innate immune defense system. However, *H. pylori*-induced autophagy is not consistent during acute and chronic infection. Therefore, a deeper understanding of the association between *H. pylori* infection and autophagy in gastric epithelial cells could aid the understanding of the mechanisms of persistent infection and the identification of autophagy-associated therapeutic targets for *H. pylori* infection. The present review describes the role of *H. pylori* and associated virulence factors in the induction of autophagy by different signaling pathways during acute infection. Additionally, the inhibition of autophagy in gastric epithelial cells during chronic infection was discussed. The present review summarized *H. pylori*-mediated autophagy and provided insights into its mechanism of action, suggesting the induction of autophagy as a novel therapeutic target for persistent *H. pylori* infection.

Contents

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1. Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative, spiral, flagellated, microaerophilic bacterium that was identified in 1983 (1). Among the global population, ~50% are chronically infected with *H. pylori*, resulting in various symptoms, including gastritis, peptic ulcer and increased neoplastic disease, as well as adenocarcinoma and mucosal-associated lymphoid tissue lymphoma (2). In addition, the World Health Organization defines *H. pylori* as a class 1 carcinogen (3). Eradication of *H. pylori* may reduce the incidence of gastric cancer by ~3-fold (4,5).

In the past years, the efficacy of conventional therapy for *H. pylori* has decreased (6). *H. pylori* is highly resistant to metronidazole (76.3%), and moderately resistant to clarithromycin (44.9%) and dual clarithromycin and metronidazole (33.3%) (7). In the USA, the resistance rates for metronidazole, clarithromycin and ciprofloxacin have been estimated to be 79.4, 70.6 and 42.9%, respectively (8). The mechanisms underlying *H. pylori* multi-drug resistance include gene mutation, virulence genes and host immunologic tolerance (9,10). Additionally, the presence of different types of virulence factors, notably vacuolating cytotoxin (VacA) and cytotoxin-associated gene A (CagA), can result in gastric cancer carcinogenesis (11,12). Several mechanisms have been attributed for *H. pylori* resistance considering various associated processes and factors, including autophagy, apoptosis, reactive oxygen species (ROS) and proinflammatory responses (13-15).

Autophagy is upregulated to maintain cytosolic homeostasis when the innate immune defence recognizes invasive bacterial pathogens (16). However, autophagy can be upregulated or downregulated in gastric epithelial cells during *H. pylori* infection (17,18). The present review focused on the molecular mechanisms currently considered to be associated with *H. pylori*-mediated autophagy. The hypothesis that the induction of autophagy can be a novel therapeutic target for persistent *H. pylori* infection was presented.

2. Autophagy

The 2016 Nobel Prize in Physiology or Medicine was awarded to Yoshinori Ohsumi, who first illustrated that nutrient deficiency induced extensive autophagy in yeast

cells in 1992 (19). Autophagy is defined as the segregation of organelles and cellular components within double membrane vacuoles called autophagosomes (20). The fusion of autophagosomes and lysosomes generates autophagolysosomes, which degrade cytoplasmic contents (20). Therefore, autophagy can be stimulated as an intracellular defence mechanism to eliminate pathogens following their recognition by the innate immune system (21). Autophagosomes can deliver pathogens to lysosomes. Furthermore, autophagolysosomes can degrade pathogens for cellular homeostasis (20,21).

Autophagy is classified into canonical and non-canonical autophagy (22). The process of autophagy is divided into several steps, including signal induction, membrane nucleation, cargo targeting, phagophore elongation, autophagosome formation, fusion with the lysosome, cargo degradation and nutrient recycling (23). The Unc-51-like kinase 1 (ULK) complex [ULK1, ULK2, autophagy related (ATG)13, ATG101 and RB1 inducible coiled-coil 1] is essential for initiation during canonical autophagy (24). The ULK complex recruits the PI3K complex (ATG14L, phosphatidylinositol 3-kinase catalytic subunit type 3, beclin-1 and phosphoinositide-3-kinase regulatory subunit 4) to produce phosphatidylinositol 3-phosphate [PI(3)P] for the phagophore membrane nucleation step (25). Subsequently, PI(3)P binds to WD repeat domain phosphoinositide-interacting (WIPI)1, WIPI2, ATG5, ATG12 and autophagy related 16 like 1 (ATG16L1) to elongate the phagophore (23). The ATG5-ATG12-ATG16L1 complex conjugates microtubule-associated protein light chain 3-phosphatidylethanolamine for autophagosome formation (26). Finally, autophagosomes fuse with lysosomes to degrade cytoplasmic components (27).

Non-canonical autophagy is another type of ATG7- and ATG3-independent autophagy, which has been described during the development of the Drosophila midgut (28). Non-canonical autophagy has also reported as an ATG5-independent signaling pathway of autophagy (29). The non-canonical process of autophagy does not occur from a double-membrane autophagosome and is called LC3-associated phagocytosis (LAP) (22,30). LAP promotes phagosome maturation and lysosomal fusion (31).

Autophagy not only eradicates pathogens, but also serves a dual role in carcinogenesis. In 1980, a study demonstrated that the process of autophagy could be induced in leukemic cells following treatment with an antiproliferative drug (32). Our previous studies indicated that matrine had potent antitumour activity against gastric cancer cells (33,34). Autophagy is upregulated in gastric cancer cells during this antitumour process, and autophagy acts as a cytoprotective mechanism to overcome lethal stress (33). Additionally, combination treatment with matrine and autophagy inhibitors can enhance the antitumour effect of matrine in gastric cancer (34). Our previous study further demonstrated that matrine exhibited antitumour activity and induced autophagy in hepatocellular carcinoma cells (35). The extensive activation of autophagy induces autophagic cell death (35).

3. Bacteria and autophagy

Numerous pathogens can be degraded by autophagy, including bacteria, such as *Mycobacterium tuberculosis* (*M. tuberculosis*), *Listeria monocytogenes* (*L. monocytogenes*), *Francisella tularensis* (*F. tularensis*), *Legionella pneumophila* (*L. pneumophila*), *Coxiella burnetii* (*C. burnetii*), *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*), *Brucella abortus* (*B. abortus*) and *Salmonella Typhimurium* (*S. Typhimurium*) (36-43). Specific bacteria, including *M. tuberculosis* and *L. monocytogenes*, can induce the process of canonical autophagy (36,37). LRG-47 has been proposed as the only agent specifically active against *M. tuberculosis* (44). LRG-47 is involved in interferon-dependent autophagy, which can suppress intracellular survival of *M. tuberculosis* (36). *L. monocytogenes* interacts with the protein internalin K (InlK), a member of the internalin family of proteins specific to *L. monocytogenes* that interact with the major vault protein (MVP) (37). MVP recruitment prevents the autophagic recognition of intracellular bacteria, leading to an increased survival rate of InlK-overexpressing bacteria (37). Canonical autophagy can restrain the growth of intracellular bacterial species. These intracellular bacterial pathogens evade intracellular defence mechanisms of host cells by escaping from the autophagosome and by modulating canonical autophagy (45). Specific bacteria can generate the process of non-canonical autophagy, including *F. tularensis, L. pneumophila*, *C. burnetii*, *Y. pseudotuberculosis*, *B. abortus* and *S. Typhimurium* (38-43). *F. tularensis* can induce ATG5-independent autophagy, which provides nutrients that support bacterial proliferation (38). *B. abortus* ensures its persistent survival by forming the Brucella-containing vacuole (BCV) (46). BCV formation is independent of the autophagy proteins, namely ATG5, ATG16L1, autophagy related 4B cysteine peptidase, ATG7 and protein light chain 3B (42). Non-canonical autophagy may be beneficial to the infectivity and growth of intercellular bacteria (38). Although *H. pylori* is an extracellular pathogen, it can also reside and grow in gastric epithelial cells, causing persistent infection (47). The first observation of autophagy was reported for a cytotoxin of *H. pylori* in 1992 (48). Subsequently, it has been verified that *H. pylori* invasion of the gastric mucosa can trigger canonical rather than non-canonical autophagy (17,49,50).

4. Acute infection of *H. pylori* can induce autophagy

A physiological mechanism of outer membrane vesicles (OMVs) from bacteria can deliver peptidoglycans into the host cell cytosol and induce an immune response *in vivo* (51). OMVs from *H. pylori* can induce autophagy, which is essential for proinflammatory chemokine production (52). OMVs rely on the nucleotide-binding oligomerization domain-1-receptor interacting serine/threonine kinase 2 signaling pathway, which is essential for the induction of autophagy and the production of interleukin 8 (52,53). In addition, *H. pylori* OMVs induce autophagosome formation, which is not dependent on VacA (52). *H. pylori* secretes HP0175, which has been identified as an inducer of apoptosis in gastric epithelial cells (54). HP0175 can also upregulate the expression of autophagy-associated genes independent of functional VacA during acute infection (17).

VacA is a critical virulence factor involved in the pathogenesis of peptic ulceration and gastric cancer (55). The toxins of VacA can induce a series of intracellular alterations, including cell vacuolation, membrane channel formation,

disruption of endosomal/lysosomal function, apoptosis and immunomodulation (56). VacA localizes in the mitochondria and induces their dysfunction (57). VacA relies on the inhibition of rapamycin complex 1 (mTORC1), which coordinates nutrients and energy stress signals in order to promote metabolic homeostasis (58). In VacA-intoxicated cells, the VacA-dependent inhibition of mTORC1 signaling results in the activation of cellular autophagy via the ULK1 complex (59). Low-density lipoprotein receptor-related protein-1 (LRP1) is the receptor for VacA-induced autophagy (60). VacA forms LRP1 conjugates in order to regulate the formation of autophagosomes and autolysosomes (60). Additionally, VacA can induce autophagy via endoplasmic reticulum (ER) stress (61). Inhibition of autophagy can decrease VacA-induced cell death in AGS cells (61). Tribble pseudokinase 3 (TRIB3) serves an important role in ER stress-induced autophagy (61,62). VacA can trigger ER stress and increase the expression of TRIB3 in AGS cells (61). Knockdown of the ER stress effector protein can significantly decrease the formation of autolysosomes and cell death (61). Therefore, VacA causes autophagic cell death via ER stress in gastric epithelial cells. Additionally, VacA-induced autophagy can degrade the toxins and limit host cell damage, leading to the maintenance of cellular homeostasis (63). VacA-induced autophagy does not affect the formation of VacA-large vacuoles (49).

The CagA protein is the fourth most abundant protein of *H. pylori* (64). This bacterium uses the Cag type IV secretion system to release CagA into host cells (65). CagA can induce multiple cellular activities, such as cytosolic vacuolation, mitochondrial dysfunction, ER stress, and endosomal stress, resulting in tissue inflammation (66). Intracellular CagA does not persist in the AGS cell line (67). VacA can reduce glutathione levels and bind to LRP1 to enhance Akt phosphorylation and activate autophagy, leading to CagA protein degradation (60,68). Intracellular CagA is degraded by autophagy induction caused by the accumulation of ROS, suggesting that CagA may not promote carcinogenesis (68). Due to the resistance of ROS, CD44-positive gastric cancer stem-like cells increase the expression levels of CagA by inhibiting autophagy (68,69). Sulfasalazine can prevent the accumulation of CagA in CD44-positive cells by upregulating autophagy, suggesting a prophylactic effect of this compound on CagA-dependent gastric cancer development (68). Overall, *H. pylori* may disturb homeostasis in host cells during acute infection. This effect has been noted in the VacA+ or Cag+ *H. pylori* strains (60,61,68). Autophagy, which targets intracellular bacteria to restrict their growth and survival, is an important defence mechanism for gastric epithelial cells (Fig. 1).

5. Chronic infection of *H. pylori* can inhibit autophagy

The mechanisms of chronic infections of *H. pylori* are not the same as those described for acute infection. MicroRNA (miR)-30b is upregulated during chronic *H. pylori* infection (70). Beclin-1 (*BECN1)* and *ATG12* are targets of miR-30b, and inhibit autophagosome formation (71). Compromised autophagy promotes persistent infection of *H. pylori* (71). Additionally, *ATG2B*, *ATG5*, *ATG12*, *BECN1* and BCL2 interacting protein 3 like are targets of miR-30d, leading to the repression of autophagy (72). miR-30d further promotes the intracellular survival of *H. pylori* during chronic infection (72). Furthermore, *H. pylori* infection induces methylation silencing of microtubule associated protein 1 light chain 3α variant 1, which may impair autophagy and facilitate gastric carcinogenesis (73). With regard to chronic infection and *H. pylori*-induced autophagy *in vitro* and *in vivo*, a consensus has been reached. A total of 28 autophagic genes are significantly downregulated in *H. pylori* GC026-challenged AGS cells (74). Previous findings derived from the human autophagy database and published microarray data demonstrated that the core autophagic genes (*ATG16L1*, *ATG5*, *ATG4D* and *ATG9A*) are downregulated in patients with chronic *H. pylori* infection with mild dyspeptic symptoms (75).

Exposure to VacA for prolonged periods may mimic the chronic infection model of VacA+ *H. pylori* strains (76). Autophagy is disrupted by the prolonged co-culture of VacA, since cathepsin D expression is inhibited in autophagosomes (18). Therefore, VacA can further inhibit autophagy in gastric epithelial cells during chronic infection of *H. pylori*.

In addition, CagA+ *H. pylori* strains can persistently reside in gastric mucosal tissues (77). The expression levels of autophagic proteins are downregulated by the c-Met/Akt signaling pathway, whereas the production of the inflammatory cytokines is upregulated in the CagA+ *H. pylori* patients (78). Therefore, several signaling pathways can induce the downregulation of autophagy as a result of chronic infection of *H. pylori* (Fig. 2). To the best of our knowledge, the role of inflammation in *H. pylori*-induced autophagy dysfunction remains largely unknown. Luo *et al* (79) demonstrated that autophagy is required for hepatitis B virus X protein-induced NF-κB activation, and for pro-inflammatory cytokine production. Dysregulated autophagy causes activation of NF-κB, which can stimulate the inflammatory response (80). This process is manifested by upregulation of cytokines and chemokines and by the inflammatory cell infiltration of the pancreas (81). Notably, the combination of sialic acid and catechins can upregulate autophagy and downregulate apoptosis in order to protect against *H. pylori*-induced gastric injury (15,82). Additionally, rapamycin can increase the clearance of *H. pylori* by upregulating autophagy (83). The inducers of autophagy may be novel therapeutic antibiotics that can be used for the treatment of chronic *H. pylori* infection.

6. Conclusions

Our previous studies demonstrated that autophagy exhibited a cytoprotective function in cancer cells and that it could induce autophagic cell death at different stages of cancer formation (33-35). *H. pylori* may disturb homeostasis in host cells during acute infection, notably via the secretion of virulence factors. Autophagy is an important defence mechanism that can restrict bacterial survival and growth. Gastric epithelial cells can induce canonical autophagy in order to maintain homeostasis during acute infection with *H. pylori*. Chronic infections with *H. pylori* can cause the dysfunction of autophagy-associated proteins. The inhibition of autophagy can lead to persistent infection. *H. pylori* can resist antibiotic treatment, and, as a consequence, the chronic infection of this bacterial strain has become a global health

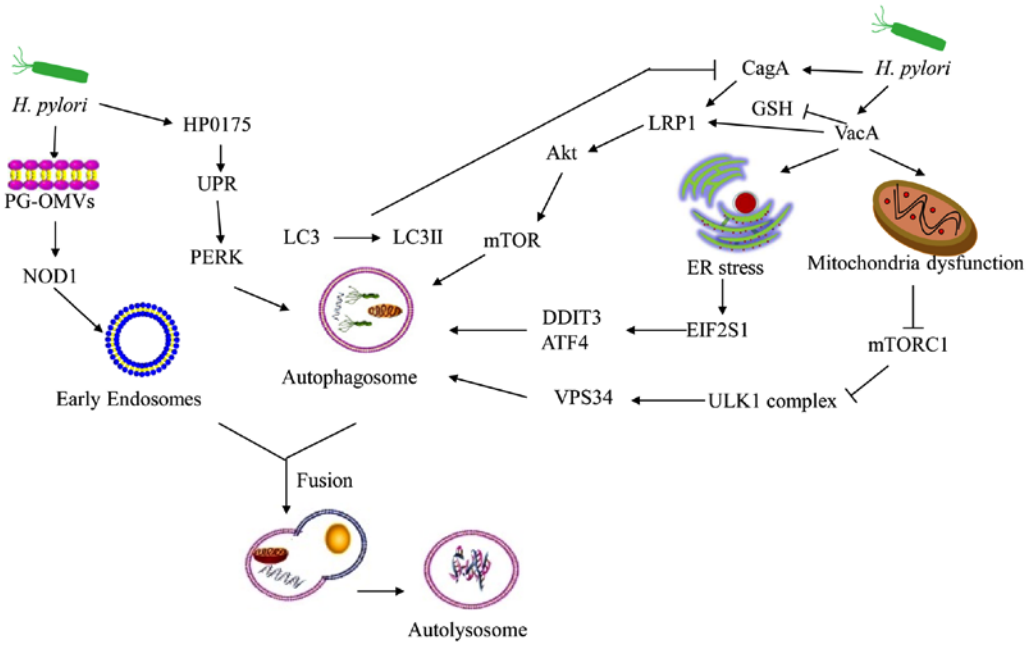

Figure 1. Acute infection of *H. pylori* can induce autophagy. *H. pylori*-OMVs stimulate NOD1, thereby triggering an autophagic response. *H. pylori* secretes HP0175, which upregulates UPR-dependent autophagy. VacA inhibits mTORC1, subsequently activating cellular autophagy via the ULK1 complex. VacA forms conjugates with LRP1 to regulate the formation of autophagosomes and autolysosomes. VacA can also induce autophagy via induction of ER stress. VacA can reduce GSH levels to bind LRP1 and subsequently enhance Akt phosphorylation to activate autophagy, causing CagA degradation. VacA, vacuolating cytotoxin; CagA, cytotoxin-associated gene A; LRP1, low-density lipoprotein receptor-related protein-1; ER, endoplasmic reticulum; EIF2S1, eukaryotic translation initiation factor 2 subunit 1; DDIT3, DNA damage-inducible transcript 3; ATF4, activating transcription factor 4; ULK1, Unc-51-like kinase 1; UPR, unfolded protein response; PERK, PKR-like ER kinase; PG-OMVs, peptidoglycan-outer membrane vesicles; NOD1, nucleotide-binding oligomerization domain-1; LC3, microtubule-associated protein light chain 3; GSH, glutathione; *H. pylori, Helicobacter pylori*; mTORC1, mTOR complex 1; VPS34, phosphatidylinositol 3-kinase catalytic subunit type 3.

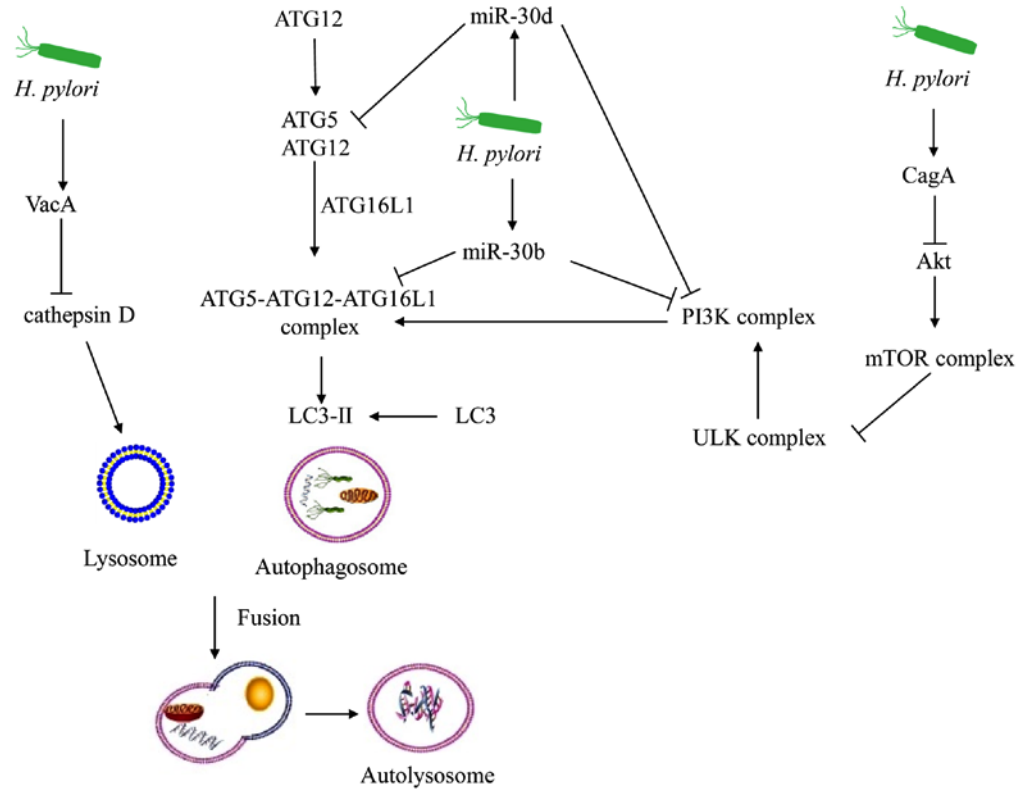

Figure 2. Chronic infection of *H. pylori* can inhibit autophagy. Autophagy-associated proteins are the targets of miR-30d and miR-30b, inhibiting autophagosome formation. Autophagy is disrupted by the prolonged co-culture of VacA since cathepsin D expression is inhibited in autophagosomes. The expression of the autophagic proteins is downregulated by the Akt signaling pathway during chronic CagA+ *H. pylori* infection. VacA, vacuolating cytotoxin; CagA, cytotoxin-associated gene A; ULK, Unc-51-like kinase 1; LC3, microtubule-associated protein light chain 3; ATG5, autophagy related 5; ATG12, autophagy related 12; ATG16L1, autophagy related 16 like 1; mir-30b, microRNA-30b; mir-30d, microRNA-30d.

issue. During infection, the induction of autophagy, which maintains cellular homeostasis, is inhibited. By upregulating autophagy-associated proteins in gastric epithelial cells, *H. pylori* can be eliminated. This strategy can be applied with the use of autophagy inducers as novel therapeutic agents. Although the mechanism of multi-drug-resistance acquired by *H. pylori* relies on associated virulence factors to cause downregulation of autophagy and maintenance of persistent infection, the precise identification of the proteins involved in this signaling pathway remains unclear.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

YL contributed to the planning and design of the study. FZ, CC, JH, RS, JZ, ZH, HC and YL were responsible for the literature search and the writing of the manuscript. FZ and YL performed revisions of the manuscript. All authors have read and approved the final manuscript for publication.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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