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Current information on the association of *Helicobacter pylori* with autophagy and gastric cancer

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**Abstract**

*Helicobacter pylori* (*H. pylori*) is a Gram-negative bacterium and causative agent of gastric cancer. *H. pylori* induce defective autophagy or inhibit it by means of CagA and vacuolating cytotoxin A (*VacA*) toxins leading to the gastric cancer induction. Impaired or defective autophagy leads to the accumulation of cytotoxic materials, such as ROS and P62 that lead to increased mutations in the DNA, genome instability, and risk of cancer formation. *H. pylori* CagA may inhibit autophagy through the c-Met-PI3k/Akt-mTOR signaling pathway. However, *VacA* induces autophagy by some signaling pathways. In the gastric epithelial cells, *VacA* is a necessary and sufficient factor for the creation of autophagy. While CagA is a negative regulator of this phenomenon, the elimination of this gene from *H. pylori* has increased autophagy and the production of inflammatory cytokines is reduced. In gastrointestinal cancers, some of the microRNAs (miRNAs) act as tumor suppressors and some other are oncogenes by regulating various genes expression. *H. pylori* can also modify autophagy through a mechanism that includes the function of miRNAs. In autophagy, oncogenic miRNAs inhibit activation of some tumor suppressor signaling pathways (e.g., ULK1 complex, Beclin-1 function, and Atg4 messaging), whereas tumor suppressor miRNAs can block the activation of oncogenic signaling pathways. For instance, Beclin-1 is negatively regulated by miRNA-376b (oncogenic miRNA) and miRNA-30a (tumor suppressor miRNA). Similarly, Atg4 by miRNA-376b (oncogenic miRNA) and miRNA-101 (tumor suppressor miRNA). So, this apparent paradox can be explained as that both Beclin-1 and Atg4 play different roles in a particular cell or tissue.

**KEYWORDS**
autophagy, gastric cancer, *Helicobacter pylori*, miRNAs

1 | EPIDEMIOLOGY OF GASTRIC CANCER

Gastric cancer (GC) is the fifth most common cancer in the world after lung, breast, colorectal, and prostate cancers. Approximately, two-thirds of the gastric cancer cases occur in East Asia, Eastern Europe, and South and Central America. The prevalence of GC in developing countries includes over 80% in adults and in developed countries, 20% of the population in thirty years old and 50% of the elderly population. (Eusebi, Zagari, & Bazzoli, 2014). In developing countries, a high percentage of children are infected until the age of 10, while in developed countries this percentage is low. The occurrence of infection is sex-independent and increases with age.
Although the transmission routes of infections are not definitely known, epidemiological studies further explore the possibility of person-to-person transmission. Transmission by animals, water, food, and endoscopy has also been considered (Guo, Zhao, & Zhang, 2011; Figure 1).

*H. pylori* is one of the most successful human pathogens that are colonized (in gastric) in half of the world’s population and remain in the body throughout the host’s life (Goh, Chan, Shiota, & Yamaoka, 2011). Colonization of bacteria in gastric mucus occur during childhood and around 10% of the people are infected with gastritis and gastric ulcer to MALT lymphoma and gastric cancer (Saito et al., 2012). Genetic and phylogenetic studies have made it clear that the bacterium has been associated with humans for nearly 88,000 years and has been isolated from all parts of the world. The current geographical distribution of *H. pylori* alleles has revealed the ancient migrations of human populations. Using polymerase chain reaction (PCR) assay, *H. pylori* is detectable in the dental plaques and saliva of children and adults but PCR does not show the presence of live bacteria. The oral route is likely among mothers who chew the food for infants. The presence of bacteria in feces with cultures has been proven, but it is difficult to prove the presence of *H. pylori* in feces with PCR. Oral transmission is likely to be most common in developed countries, but the fecal-oral transmission is more common in developing countries. The high prevalence of this bacterium in mentally retarded patients has led to fecal-oral transmission of *H. pylori*. *H. pylori* are also isolated from cases of cardiac endocarditis patients (Bauer et al., 2011; Portal-Celhay & Perez-Perez, 2006; Rokkas, Sechopoulos, Pistiolas, Margantinis, & Koukoulis, 2010; Sanchez-Delgado et al., 2011; Figure 2).
Except for the above mentioned socioeconomic factors, such as health, poverty, population congestion, water pollution, and individual behaviors (e.g., chewing food by mother for infants) are among the most effective factors of contamination in the most of the developing countries. Moreover, in some studies *H. pylori* have been isolated from cats, suggesting the possibility of transfer from pets to humans and vice versa (Bauer et al., 2011; Figure 3).

**2 | PATHOGENESIS OF *Helicobacter pylori***

*H. pylori* pathogenesis occurs due to its unique survival strategies and the ability to escape the host’s immune system. To achieve this goal, *H. pylori* has been equipped with a number of evolved virulence factors including two most important factors: Vacuolating cytotoxin A (*VacA*) and an active protein associated with cytotoxin gene A (*CagA*). In the clinical strains, the *CagA* and *VacA* are associated with the severity of infection and thus they are divided into type I (with an expression of both *CagA* and *VacA* genes) and type II (not expressing the *CagA* and *VacA*; Siao & Somsouk, 2014).

Although *H. pylori* is an extracellular pathogen, it can remain and proliferates in the macrophage autophagosomes, gastric epithelial cells, and dendritic cells. Infection with *H. pylori* leads to the innate immune responses for production of antimicrobial peptides, inflammatory regulators, and various free radicals of reactive oxygen species (ROS; Testerman & Morris, 2014). Cytokines are released by epithelial cells into the lamina propria, which activate macrophages, dendritic cells, and other inflammatory mediators. This response then activates the primary T lymphocytes, regulatory T lymphocytes, B lymphocytes, and neutrophils. Almost all patients with *H. pylori* suffer from chronic gastritis. However, only 10–15% develop gastric ulcers and 1–3% gastric cancer (Molnar, Galamb, Sipos, Leiszter, & Tulassay, 2010). Therefore, there is a complex integration between the host, the microenvironment, and bacterial factors that can affect the carcinogenicity of the infection. In recent years, it has been revealed that *H. pylori* can affect the autophagy pathway of the host, a protected process in which cytosolic proteins, organs, and pathogens are isolated, digested, and degraded (Handa, Naito, & Yoshikawa, 2010; Hayashi et al., 2012). Recently published studies

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**FIGURE 3** Macroautophagy, microautophagy, and chaperone-dependent autophagy and their contribution to the digestion of proteins and intracytoplasmic organelles: (a) macroautophagy activity with a regulatory complex (including Vps34, Beclin 1, Vps15, Ambra1, and Atg14) that causes the LC3 protein recruitment into the initial autophagosome (detached from the membrane). Selective degradation by mitochondria (mitophagy) is one of the specific mechanisms of macroautophagy that requires the PINK1-parkin protein complex and Bnip3 factors. Proteins that are committed to lysosomal degradation (including BAG3 and filamin) are tagged by the ubiquitin chains and transmitted to autophagosomes by the p62 protein. (b) Micropathophysiology involved in the direct ingestion of small proteins inside the cytoplasm into the lysosomes. (c) In the CMA, proteins affected by various factors, such as free oxygen radicals, exhibit specific amino acid sequences (called KFERQ motifs) recognized by the Hsc70 chaperone and transmitted into the lysosome via the Lamp2a receptor. CMA: chaperone-mediated autophagy; LC3: light chain 3 [Color figure can be viewed at wileyonlinelibrary.com]
have revealed that VacA can alter host pathways, which may have important implications for *H. pylori* carcinogenesis. The most important cellular mechanisms exacerbating the infection include endoplasmic reticulum (ER) stresses; unfolded protein response, autophagy, oxidative stress, and inflammation. The relationship between autophagy and ER stress has been well documented. In case of *H. pylori*, it has been shown that the HP0175-secreted antigen can regulate PERK, which, in turn, activates ATF4 and CHOP transcription factors, which induces autophagy in the gut epithelial cells (Chen et al., 2016; Hoy et al., 2010).

3 | AUTOPHAGY IN *H. pylori* INFECTION AND ITS ASSOCIATION WITH GASTRIC CANCER

Autophagy means eating itself, a process in which cellular proteins, organelles, and intracellular pathogens are isolated within a crescent of the membrane, length of which gradually increases with two layers of membrane, referred to as autophagosome. Fusion of autophagosome into the lysosome forms an autophagolysosome that brought degrading enzymes into the compartment and consequently resulted in the degradation of the contents of the autolysosome. This process can be selective or nonselective. Nonspecific autophagy increases under stress and hunger and involves the destruction of a significant number of cytosolic components to provide nutrients. In contrast, the selective autophagy includes degradation of damaged organs or invading pathogens, which are usually followed by a “label” (e.g., ubiquitin) to identify labeled cargo for autophagic degradation (L. Zhang et al., 2018).

Autoptosis causes by chemical stimulators, such as hypoxia, energy depletion, temperature, hormones, drug factors, cytokines, and disease progression. In contrast to incomplete autophagy, it refers to the pathogenesis of cancers, aging, neurological damage, infectious diseases, and inflammatory bowel disease. In carcinogenesis, the tumor suppressor function of autophagy is performed through the destruction of damaged organelles, reduction of active ROS agents, and increase the genomic stability (Han et al., 2015). In addition, ROS seems to have a mechanical role in autophagy. Studies in adenocarcinoma gastric cell line (AGS) cells have revealed that ROS plays a key role in the induction of autophagy, which can be prevented by adding antioxidant N-acetylcysteine. In contrast, the use of an NADPH oxidase (NOX) inhibitor or similar mitochondrial superoxide dismutase cannot prevent VacA-induced autophagy, indicating that produced NOX and ROS by mitochondria were not involved in autophagy in this pathway. The apparent increase in ROS in *H. pylori* infection can lead to decreased intracellular glutathione levels with an unknown mechanism through VacA (Kato, Tsugawa, Saito, & Suzuki, 2018).

Autophagy plays a pivotal role in host defense against foreign microbes. Defective autophagy also increases the bacterial growth in the intestine. Some pathogens may disrupt the autophagy system to grow and survive. For example, it reduces the intracellular survival of *Shigella flexneri* and *Salmonella enterica*. *S. flexneri*, and *Listeria monocytogenes* have evolved various mechanisms and can disrupt autophagosomes. Some pathogens modulate the intracellular trafficking and suppress the autophagosomes, like *Legionella pneumophila*, which inhibits autophagosome maturation. Several studies also discuss the role of autophagy in clearing microbial toxins and reducing their effects. Like *Vibrio cholerae* cytolsin and *Staphylococcus aureus* α-hemolysin, which play an important role in bacterial survival and can be found in the membrane of autophagosomes (Brothers, Kowalski, Tian, Kinchington, & Shanks, 2018).

Autophagy is a protected lysosomal refraction pathway that degrades cytoplasmic content and is important in host cell defense. It is essential for survival, differentiation, and cellular development and plays an important role in host safety and homeostatic maintenance. Three distinct paths have been identified in autophagy including (a) macroautophagy, (b) chaperone-mediated autophagy (CMA), and (c) microautophagy.

4 | MACROAUTOPHAGY

Macroautophagy is a conventional pathway and the most well-known pathway in which the outer membrane autophagosome fuses with a lysosome to discharge its own content in an autolysosome. Macroautophagy is activated by a set of regulatory molecules (containing Vps34, Beclin-1, Vps15, Ambra1, and Atg14), which causes light chain 3 (LC3) to be absorbed into the nascent autophagosome (isolation membrane). For selective removal, of mitochondria (mitophagy) requires the complex of PINK1-parkin and the Bnip3 factor. Proteins that are committed to lysosomal degradation (including BAG3 and filament) are labeled with polyubiquitin chains and are delivered by the p62 protein scaffold (Xiao et al., 2017).

The transfer of autophagy studies from morphology to molecular machines was based on the identification of Atg genes and led to the identification of >30 genes from Atgs. Among these Atg genes, a subgroup contains approximately 18 genes among different types including nonselective macroautophagy, cytoplasm pathway to vacuolating (a similar biosynthetic pathway), mitophagy, and pexophagy. Specifically, the products that are associated with this subtype gene are needed for the formation of autophagy and are the main components. These autophagy nucleus proteins can be subdivided into the different functional groups including (a) Atg1/ULK (Atg1, Atg11, Atg13, Atg17, Atg29, and Atg31), which are involved in regulating induction of autophagosomes; (b) Atg9 and its cycling system (Atg2, Atg9, and Atg18), which after the assembly of the Atg1 complex in PAS, plays an important role in delivering the membrane to expanded phagophore after the formation of the Atg1 and PAS complexes; (c) PtdIns 3-kinase (PtdIns3K) Vps34 complex, Vps15, Vps30/Atg6, and (Atg14) in the vesicle nucleation stage that contributes to the application of the PtdIns3P binding proteins to the PAS; (d) two ubiquitin-like (Ubl) ubiquitin systems for the conjugation of Atg5 (Atg12: Atg7), Atg10, Atg12, Atg16, Atg3, Atg8, Atg4, Atg7,
and Atg8 to develop the vesicles (Dunn, 1994; Yang & Klionsky, 2010). The Atg5-Atg12-Atg16L1 complex coupled with the protein associated with microtubule (a light chain of LC3) and production of a complex that is needed for the formation and development of autophagosomes. The conjugation of this complex with the autophagic membrane leads to retrieval of other autophagy proteins helping maturation. LC3 fuses in the cytoplasm and with the induction of autophagy, the LC3 is altered. The LC3-II is a lipid which is localized to the internal and external membranes of autophagosomes. In mammalian cells, LC3-II is considered as a standard for autophagic activity and as a gold marker. Among the Atg genes, the Atg16L1 gene is located on chromosome 2q37.1, which encodes a protein containing 580-630 amino acids and include an N-terminal region, which interaction of this Atg with conjugated Atg5-Atg12 and the coiled coil domain, which is an Atg16L1 dimerization mediator. Defective autophagy occurs in monocytes, where individuals of polymorphism have Atg16L1 autophagy genes that are susceptible to Crohn's disease (CD) development with alleles that increase the acquired susceptibility to *H. pylori* infection. It has been reported that the presence of known CD risk factor, Atg16L1 rs2241880, increases the risk of *H. pylori* infection in German and Scottish white people (Bonaldo & Sandri, 2013).

5  |  CMA

Proteins that are damaged by various factors, such as ROS, reveal a certain sequence of amino acids (KFERQs) that are known by the Hsc70 chaperone, which, in turn, are interacting with their Lamp2a receptors to lysosomes.

One of the distinctive features of the CMA is that in this pathway proteins are individually selected through a process for detecting amino acid sequence motifs. This allows the system to remove damaged and abnormal proteins without disturbing the neighboring proteins or even a protein from a multiprotein structure. In addition, this selective mechanism allows the CMA to play a supervisory role in multicellular processes by helping to modulate the levels of intracellular enzymes, transcription factors, and proteins contributing to cell survival. CMA is a multistage process that involves: (a) identifying the substrate and targeting the lysosomal; (b) bonding the substrate and unfolding; (c) displacing the substrate; and (d) degradation of the substrate in the lysosomal lumen. Identification of substrate proteins in cytosol occurs by coupling a constitutive, a 70KDa shock-cognate protein (hsc70), into a repeat amino acid sequences occurring in all CMA substrates (Kaminskyy & Zhivotovsky, 2012; Mizushima, 2005).

6  |  MICROAUTOPHAGY

Microautophagy involves the direct absorption of small proteins from the cytoplasm to the lysosomes. This generalized, selective and lysosomal digestive process takes place using autophagic tubes, both of which are interstitial reactions, and invagination and scission is performed inside and in the lumen. With its constructive features, the soluble substrate microdegradation can be triggered through nitrogen and/or rapamycin poverty through complex regulatory signaling pathways. The main function of microautophagy is to maintain the size of cytoplasmic organelles, membrane homeostasis, and cell survival under nitrogen restriction. In addition, microautophagy, macroautophagy, and chaperone-mediated autophagy and other phagocytic pathways have been coordinated with each other. Three forms of the selective microtubule, such as microexophagy and microautophagy of the nucleus fragmentation are activated under certain conditions (Mijaljica, Prescott, & Devenish, 2011; Sahu et al., 2011).

7  |  THE ROLE OF AUTOPIHAGY IN CANCER

Evidence suggests that autophagy has a particular role in cancer, which can have either tumor suppressive or tumor promoting activities. Autophagy has an important role in reducing inflammation, tissue damage, and genome instability as factors that affect cancers. Cells with impaired autophagy result in the accumulation of ROS and P62 chaperons and damaged ER and mitochondria. This phenotype is associated with cells that are no longer able to maintain intracellular homeostasis by eliminating damaged proteins and organs (Kondo, Kanawa, Sawaya, & Kondo, 2005).

Inevitably, impaired and defective cells in autophagy result in accumulation of cytotoxic materials, which leads to increased mutations in the DNA that enhance cancer formation. Autophagy function in tumor-suppressing is directly related to the mutation of genes that are associated with autophagy or single-allelic elimination in many cancers. For example, the removal of the monoallelic BECN1 gene in breast, ovarian, prostate, and other cancers have been reported. However, in some cases, autophagy can also enhance the risk of developing a tumor (Singletary & Milner, 2008). The microenvironmental control of the tumor typically has nutrient, oxygen, and growth factors (ischemia) deficiencies. In these conditions, regulated autophagy can increase the survival of tumor cells. Increasing autophagy in tumor cells also prevents the accumulation of nonfunctional mitochondria that can disrupt tumorigenicity. In fact, for this reason, autophagy is needed to induce a tumor triggered by oncogene Ras. Although inflammation, ROS, and DNA damage are all parts of the factors involved in the development of the tumor, they can also be harmful to the tumor itself. Therefore, autophagy may increase the survival of the tumor by limiting inflammation, ROS, and DNA damage. On the basis of the published studies, the antitumor role of autophagy and the molecules involved in this process have been somewhat specified including Atg4C, Bif-1, and UVRAG that are tumor suppressors. Thus, identification of anti-oncogenic signaling pathways including PTEN, TSC1/2, LKB1, and P53, which stimulate autophagy is important. It has been reported that tumor cells under the chosen pressure of treatment interventions are presumably protected from autophagy, which acts as an
adaptive cellular response, leading to the chemical resistance and cancer cell survival (White & DiPaola, 2009).

Recent studies have revealed that increasing the expression of beclin-1 is associated with a prognosis for gastric cancer. Autophagy is also related to matrine protective effect, a natural substance found in Sophora flavescens. In addition, it has been revealed that interferon γ (IFN-γ) inhibits gastric carcinogenesis by inducing epithelial autophagy and T-cell apoptosis in a mouse model.

An interesting point is the relationship between IRGM polymorphism (rs 4958847 and rs 13361189) and gastric cancer in the Caucasian population, which was carried out by (Burada et al., 2012). IRGM rs 4958847 has been revealed to reduce the risk of gastric cancer in these individuals. IRGM is located on chromosome 5q33.1 and the M protein encodes the immune-associated GTPase family involved in the formation of autophagy (Kondo & Kondo, 2006).

8 | CYTOTOXIN A (CagA)

CagA is an oncoprotein and an immunodominant of 120–140 kDa encoded by the Cag pathogenicity islands and enters the host cell via the T4SS secretion system. The Cag pathogenicity island consists of about 30 genes acquired by a bacterium during an evolutionary period and inserted into the glutamate racemase gene. The bacterial strains may have lost the entire Cag pathogenesis island. CagA protein directly enters the cytoplasm of the epithelial cells through the type four secretion system, where the tyrosine domain is phosphorylated by Src kinase family and transmits the transmission pathways of the cellular signal. The initial phenotypic effect of this toxin is the morphologic deformity of the cells in vitro (Viala et al., 2004). These effects have been seen to prolong and find long cavities in the cells known as Hummingbird phenotype. The second most important effect of Cag is to induce various types of transcription factors involved in controlling essential cellular activities, such as cell proliferation through factors like c-Jun and c-Fos. The third action of this protein is the destruction of the connections between the epithelial cells that destroys cell connections and facilitates the release of nutrients for bacterial growth (Ribet & Cossart, 2015).

One of the key features of CagA is the ability to communicate with host cell kinases and to alter the tyrosine phosphorylation. The phosphorylation positions of tyrosine in CagA contain a sequence of proteins from amino acids at the carboxyl terminus including Glu-Pro-Ile-Tyr-Ala (EPIYA). On the basis of the geographical distribution of the H. pylori species that have CagA and the flanking of the amino acid sequence, four distinct types of EPIYA motifs have been identified, called EPIYA-A, B, C, and D (Nell et al., 2018).

For example, in vitro human intestinal epithelial cell transfection (AGS) with type ABCCC of CagA type H. pylori (which has three successive EPIYA-C in the C-terminus region) and can significantly transcribe multiple genes involved in the gastric cancer and when similarly compared with the type of CagA ABC can induce IL-8 production and manipulate Crk-related proteins and other proteins associated with the apoptosis pathway (through its antiapoptotic effect).

The structure of this protein in some Helicobacter spp. is different, for example, between 0 and 5 tyrosine roots for phosphorylation in different types of this protein, which causes more phosphorylation sites to cause wider cytoskeletal changes in epithelial cells. Strains with this type of CagA protein have been further isolated from patients with gastric cancer. High levels of IL-8 in epithelial cells with Cag+ strains are observed in comparison with Cag− strains. This is not due to the presence of CagA protein, but due to the compounds, such as muramyl dipeptide, which secreted by the type 4 secretion system into the cytoplasm. The deep effects of CagA on multiple intracellular pathways may have major implications, such as impaired intracellular actin transport, stimulation of inflammatory responses, and disturbance in the cellular attachment. Such activities of CagA are more involved in the development of gastric cancer (Backert, Tegtmeeyer, & Fischer, 2015).

9 | AUTOPHAGY AND CagA

The effect of the CagA on autophagy was described by Terebiznek (2009). This factor has recently been identified as a negative regulator of autophagy in gastric mucosa of patients and AGS cells infected with a VacA nonfunctional strain (VacA s1m2). As a result, CagA may inhibit autophagy through the c-Met-PI3k/Akt-mTOR signaling pathway. In addition, P62 in gastric biopsy of patients infected with CagA+ strains compared to those infected with CagA− expressing strains (Deen, Gong, Naderer, Devenish, & Kwok, 2015).

It has been found that autophagy in the gastric mucosal tissue infected with CagA+ H. pylori are produced less than the CagA− strains, which is associated with the accumulation of SQSTM1 and decreasing the expression of LAMP1. SQSTM1 is the main cargo receptor of the ubiquitin in the cells, which is transmitted by the autolysosomes and the defect in autophagy leads to the accumulation of SQSTM1. Thus, SQSTM1 plays a useful role in the production of nuclear factor-xB (NF-xB)-dependent cytokines. In vitro, the removal of CagA gene was associated with an increase in autophagic activity, a reduction in the expression of SQSTM1 and cytokines, while excessive expression of CagA caused a reduction in the autophagy response induced by starvation. Thus, the production of cytokines increases with autophagy inhibition and decreases with autophagy. The results indicate that CagA-regulated autophagy leads to the accumulation of SQSTM1, which results in the release of NF-kB-dependent cytokines (Li et al., 2017).

Removal of CagA reduces the ability of Akt kinase activity on the Ser-473 site and increases autophagy. C-Met small interfering RNA (siRNA) significantly affects CagA-mediated autophagy and reduces the levels of p-Akt, p-mTOR, and p-S6. Both the siRNA C-Met and the MK-2206 can reverse inflammatory responses. These findings suggest that CagA is likely to act as a negative regulator of autophagy in the H. pylori-induced inflammatory response, especially as inflammation and autophagy are the hallmarks of gastric malignancy. It also opens a new
treatment for gastric malignancies, especially prophylaxis (Deen, Huang, Gong, Kwok, & Devenish, 2013).

C-Met activator CagA is critical via CRPIA motif (repetitive conserve responsible for phosphorylation-independent activity) that is vital for activating the PI3k/Akt signaling pathway and the pleiotropic transcriptional responses of H. pylori infection, including activation of NF-xB for the PI3k/Akt signaling pathway via mammalian by Rapamycin target (mTOR) in autophagy being an autophagic regulator suppressing the autophagy (Deen et al., 2013).

10 | THE VacA TOXIN

VacA with 88.2 kDa induces vacuole formation in tissue culture cells. The N-terminal region of VacA (P33) and the C-terminal of VacA (P55) attach to the plasma membrane and through this way enter the host cells. This protein facilitates the formation of intracellular survival in gastric cells and increases the severity of the disease, which causes autophagy in the gut epithelial cells. VacA induced autodegradation reduces VacA levels and reduces H. pylori survival. Long-term exposure to VacA disrupts autophagy, which is performed by the cathepsin D enzyme (Ricci, Romano, & Boquet, 2011).

VacA multifunctional toxin, which is linked to the large oligomeric complex forms anionic channels. Calpain-1 activator in parietal cells secreting an acid that reduces gastric acidity. VacA affects cell death and followed by endocytosis localized in the mitochondria that induce apoptosis in gastric epithelial cells (Cover & Blanke, 2005). VacA stimulates the release of cytochrome C and retrieves protein-1 associated with dynamin by activating the pore into the mitochondrial membrane. VacA also enhances cell proliferation by modulating growth-related pathways, such as P38 and β-catenin. The intrinsic and acquired immunity of the host has been changed and stimulates the release of secretory granules from mast cells and the release of proinflammatory cytokines in monocyte and eosinophils. VacA is an immunosuppressant mediated by T and B cells and inhibits antigen supply and enhances H. pylori colonization. It promotes H. pylori intracellular survival, which modulates the host endocytic pathway and creates a proliferative niche. VacA creates vacuole structures and acts as an intracellular reservoir, followed by the infection of these vacuole structures and plays an important role in keeping the infection. These vacuoles are the late endosomal origin and are associated with the presence of markers of this late endosomal, such as Rab7, CD63, and LAMP1. The presence of these markers and the reduction of pH in H. pylori vacuoles is associated with a significant reduction in cathepsin D protease in vacuoles caused by the wild-type H. pylori (Calore et al., 2010; Ghoshal, Chaturvedi, & Correa, 2010).

11 | VacA AND AUTOPHAGY

H. pylori VacA induces autophagic cell death in gastric epithelial cells through the pathway of ER stress and can be associated with advanced vacuolation and gastric ulcer and possibly gastric cancer. Evidence suggests that autophagy interferes with VacA-induced cell death. VacA induces autophagy and increases cell death of human gastric cancer cells. Autophagy inhibition can reduce the VacA-induced cell death in AGS cells. In addition, a large number of ER is observed and phosphorylation is a subunit factor 2 that increased the inducible initiating of the eukaryotic translation (subunit I) through VacA in AGS cells, while the ER stress suppression can reduce autophagy and cell death through the destruction of the transcriptional activating factor 4 and the DNA-damage-inducible transcript 3 (Yahiro et al., 2012).

In addition, the expression of TRIB3, produced by VacA, is caused by ER stress, as well as the knockdown of TRIB3 can reduce the cell death caused by VacA. Ultimately, autophagy inhibition can reduce cell death from VacAs1m1 and apoptosis. The control of Z-VAD apoptosis had no significant effect on VacAs1m1-induced autophagy, thus the VacA caused the death of autophagic cells through ER stress in gastric epithelial cells. Autophagy is one of the most important mechanisms of intracellular defense in which several types of pathogens have different strategies for escaping lysosomal degradation or controlling and modifying autophagy in their favor. In the case of Terebiznek study (2009), H. pylori infection of the gastric epithelial cells caused short-time (6 hr) autophagy in a CagA and urease independently manner by depended to VacA virulence factor. The autophagy protects cell survival by restriction of toxin-induced cell damage (Tsugawa et al., 2012).

In the 2012 Raju study, it was shown that exposure to long-term VacA (24 hr) disrupts the antiphagocytic pathway. Defective autophagosomes have been reported to accumulate within the cells. Thus, it was revealed that P62 is a selective substrate for autophagy degradation mediated by gastric biopsy of patients infected with a toxic strain of VacAs1m1 compared with other nontoxic VacAs2m2 strain. In addition, it has been reported that the effect of H. pylori on the regulation of autophagy is associated with changes in the expression of genes associated with autophagy in gastric and macrophage cells (Raju et al., 2012). In this regard, silencing of the microtubule methylation associated with MAP1LC3A variant 1 (MAP1LC3Av1) an important protein in the autophagy process has been observed in the gastric infected with H. pylori. These studies suggest modifying the autophagy in the host, which refers to VacA as a mediator. However, the reported results provide contradictory mechanisms for the accuracy of this study, which VacA sets up as trend. Autophagy is a host defense mechanism against invasive intracellular organisms. H. pylori, by escaping from this system, increases the response to intracellular survival. The change of LC3-I cytosolic to LC3-II and localization in the autophagosome membrane is the main key to autophagy induction. Autophagy modifies the intracellular stability of VacA to limit the survival of H. pylori. Autophagy is initiated in host cells to remove microbial toxins and protects the cell against toxin-mediated damage (Raju et al., 2012).

The H. pylori VacA is a defective autophagy inducer. The bacteria trap the endocytic mechanisms of the host to create intracellular niches in large vacuoles to increase its survival. Autophagy is a key
endocytic pathway, which is typically used to get rid of the invasive pathogens. Published studies have revealed that VacA modifies autophagy in vivo and in vitro. Exposure to VacA results in the accumulation of incomplete autophagosomes and reduction of cathepsin D. Pore-forming activity of toxin is required for autophagy, such as Listeriolysin O of L. monocytogenes and V. cholerae cytolsin. Autophagy is associated with tumor suppressor functions that inhibit levels of ROS. Defective autophagy can increase ROS and cause genomic instability leading to the cancerous deformation (Palframan, Kwok, & Gabriel, 2012).

In H. pylori infection, autophagy is induced in response to the VacA and plays a protective role in the cell. Therefore, prolonged exposure to VacA is impaired by autophagy, which reduces cathepsin D by autophagosomes and decreases catalytic activity. Several studies have been conducted to determine the signaling events that initiate VacA’s autophagy. Tsugawa et al. suggested that p53, an mTOR activator, is involved in this process. The researchers have shown that ROS induces VacA to stimulate phosphorylation of AKT, followed by phosphorylase synthesis of another molecule called murine double minute 2 (MDM2), which is a major component of the E3 ubiquitin ligase involved in the destruction of P53. They showed that increased levels of MDM2 phosphorylation were associated with a reduction in p53 following H. pylori infection. Phosphorylated MDM2 levels have only increased in response to the VacA S1m1, resulting in autophagy induction alone being performed with VacA S1m1. Therefore, signaling by the destruction of P53 seems to be at least one of the mechanisms by which VacA induces autophagy in the gastric cells. Given that VacA treatment reduces the level of adenosine triphosphate, it is believed that VacA-induced autophagy may also be induced by AMPK (5′-AMP-activated protein kinase) signaling pathways (Tsugawa et al., 2012).

Several studies have already revealed that infection with H. pylori causes autophagy in both gastric epithelial cells and professional phagocytes. In the epithelial cells of the gastric, VacA is a necessary and sufficient factor for the induction of autophagy. Recently, Yahiro et al. have reported that VacA-induced autophagy caused by binding of VacA to protein 1, which is associated with the lipoprotein receptor (LRP1). This pathway has been confirmed by silencing the expression of the LRP1 gene using the internalized siRNA. In addition, the VacA-induced autophagy appears to be dependent on the intolerance of this toxin. Interestingly, only VacA S1m1 and not VacA S1m2 respond to LRP1 and play a role in autophagy and the VacAmp2 region does not interfere with VacA-LRP1 binding. The study also found that by using the siRNA and inhibition of the LRP1 expression, the LRP1-associated autophagy is independent of other VacA receptors, including receptor-like protein-tyrosine phosphatase RPTPζ, RPTPβ, and fibronectin (Yahiro et al., 2012).

### 12 | The Consequences and Changes of Autophagy in H. pylori Infection

The role of autophagy in H. pylori infection is complex and may play a significant role in various stages of infection. At first, autophagy may be used as a home defense for destroying invading bacteria. A similar role in autophagy has been found in increasing the potency of bacterial cleaning in the human gastric epithelial cancer cell line. Tang et al. showed that the addition of 3-methyladenine inhibitor resulted in more intracellular survival and proliferation of H. pylori while increasing autophagy by treatment with rapamycin or causing starvation reduced bacterial survival. This finding also applies to human macrophages. Interestingly, in both macrophage cells and dendritic cells derived from human bone marrow, VacA and CagA mutations in H. pylori resulted in faster clearance compared to their wild-type. This suggests that VacA and CagA are helpful in increasing intracellular survival and proliferation in these cells but this finding was not observed in gastric epithelial cells because VacA increased survival, but CagA did not have an effect on its survival. These differences may be attributed to the use of phagocytic and nonphagocytic cells (Posselt, Backert, & Wessler, 2013).

At first, autophagy was seemed to act as a host defense to reduce the damage caused by secreted toxins. In gastric epithelial cells, the destruction of the essential protein for Atg12 autophagy leads to an increase in VacA levels in infected cells. Tsugawa et al. also showed that VacA-induced autophagy plays an important role in intracellular CagA regulation. After the infection of AGS cells, intracellular levels of CagA are reduced by time. Most important, the level of this protein is not altered by proteasome inhibitors but is significantly increased after treatment with autophagy inhibitors (Radin et al., 2014).

### 13 | The Relationship Between VacA, ER Stress, Autophagy, and Cell Death in AGS Cells

Evidence suggests that H. pylori VacA induce autophagic cell death in gastric epithelial cells through stress-induced ER pathway. This novel mechanism derives from the following results: (a) VacA stimulates the expression of Cx43, the gap junction channel protein, which is a key regulator of cell-to-cell communication. This increased expression of Cx43 leads to the formation of ER stress, contributing to the development of peptic ulcer development in H. pylori infection. Apoptosis appears to be a critical factor in VacA’s cell death. VacA induces apoptosis through the accumulation of connexin 43 (Cx43) via Rac1 (Halder et al., 2015).

Although VacA does not affect the expression of Cx43, the accumulation of Cx43 leads to apoptotic cell death of A2-521 cells. Interestingly, autophagy appears to occur earlier in apoptosis in AGS cells. The 3MA autophagy inhibitors or the Atg12 or BECN1 knockdown can inhibit poly(ADP-ribose) polymerase cleavage, activation of caspase 3, and cell death. This data shows that autophagy increases the death rate of gastric epithelial cells under
VacA stress conditions and mediates apoptosis. Although the mitochondria are the target of VacA, how VacA affects mitochondria during apoptosis is still unclear. One study found that VacA could increase the phosphorylation of EIF-2S1 and cause knockdown of DDIT3 or ATF4 effectors in ER stress, which can significantly reduce the autolysosome and cause cell death in VacA-treated AGA cells. Akazawa et al. reported that VacA increases the transcription of C/EBP (CCAAAT/enhancer binding protein) homologous protein and EIF-2S1 phosphorylation in gastric epithelial cells and knockdowns of CHOP (C/EBP homologous protein) and inhibit the VacA-induced apoptosis. In this study, ER activation induced by VacA also helped apoptosis of gastric epithelial cells. The results of this study showed that VacA causes an ER response to activation of autophagy and causes AGS cell death (Greenfield & Jones, 2013).

miRNAs are small and noncoding RNAs of ~22 nucleotides (nt), playing a major role in regulating cellular mortality, apoptosis, and autophagy. More than 1000 miRNAs have been identified and each miRNA can regulate the expression of hundreds to thousands of target genes and it is estimated that 30–92% of human genes are regulated by miRNAs. Evidence from studies has revealed that miRNAs can affect autophagy pathways in many pathologic processes, in particular, modulates cancer. In cancer cells, autophagy is one of the most important physiological mechanisms that can be used temporarily as a means of survival, whereas cellular death occurs if cellular stress leads to continuous or excess autophagy. Therefore, autophagy may play an important role in regulating a limited number of Atg-related genes, such as Atg1 (ULK1)/Unc51, Atg4, a protein associated with Bcl-2 (Bclin-1), and LC3/Atg8, which ultimately play a decisive role in the fate of cancer cells. The importance of miRNAs and autophagy in the oncogenic signaling or tumor suppressor pathways is considered as a double-edged sword (a guard and executioner; Xu, Wang, Tan, & Jing, 2012).

Recently, miRNAs that are involved in regulation a number of Atgs members and their coordinators’ in various stages of autophagy are well known that have a role in initiating, the formation of a vesicle nucleus and elongation of the vacuole and completing it (Frankel & Lund, 2012).

In the first step, induction of autophagy begins activating the ULK1 complex, which is controlled by the mTOR (Kim, Kundu, Viollet, & Guan, 2011). miRNA-106a plays an inhibitory role that prevents ULK1 activation and thus prevents autophagy induction. Second, the formation of a vesicle depends on the complex of kinase core (called Beclin-1 phosphatidyl inositol 3 class III [PI3KClIII]-Vps15) and other proteins (Portal-Núñez, Esbrit, Alcaraz, & Largo, 2016). In this stage, miRNA-30a and miRNA-376b can inhibit Beclin-1 activity, thus preventing the formation of vacuole nucleus. In addition, miRNA-101 can inhibit RAB5A, which is a small GTPase that acts as an initial regulator for endocytosis and early formation of autophagosomes (Fu, Wen, Bao, & Liu, 2012). Third, the process of vacuole membrane elongation and completing needs to ubiquitin-like the system in which two proteins, Atg12, and LC3/Atg8, play a role. During this process, miRNA-183 and miRNA-204 can inhibit the activity of the LC3B1 and LC3-II proteins, thereby negatively regulating the process of completion of the vacuole. miRNA-101 and miRNA-376b can be negative factor respectively for the expression of Atg4C and Atg4D (Fu et al., 2012).

Overexpression of miRNA-106a, belonging to the highly oncogenic miRNA family, directly plays a role in the reduced regulation of ULK1 messenger RNA level in acute myeloid leukemia, suggests that ULK1 is a tumor suppressor. While the oncogenic miRNAs target transcriptional levels of tumor suppressor genes, mTORC1 is one of the negative regulators of autophagy that led to mAtg13 and ULK1 interruption, which makes the unstable ULK1 complex (Fu et al., 2012; Panda et al., 2015).

Similarly, miRNA-376b plays a role in autophagy reduced by Rapamycin, thereby directly decrease the translation levels of Beclin-1 and Atg4C in Hun-7 and MCF-7 cells (Fu et al., 2012).

miRNA-183, together with miRNA-182 and miRNA-96, is often overexpressed and potentially acting as an oncogene. Silencing miRNA-183 expression can cause the death of moderate thyroid cancer cells by increasing the expression of LC3B1 (LC3-I), which suggests miRNA-183 may be an attractive therapeutic target. In addition, miRNA-31 and miRNA-34c can provide protection against mitochondrial autophagy (mitophagy) under oxidative stress and act as positive regulators of cancer progression and metastasis in the stroma tumor model (Fu et al., 2012).

The first report of a miRNA associated with tumor suppressor autophagy belongs to the miRNA-30a, which is a negative regulator of the expression of Beclin-1, thereby reduce the autophagy induced by Rapamycin in T98G cell line. miRNA-101 can inhibit autophagy by targeting Atg4D, STMN1, and RAB5A, which plays the same role as Atg4C, Atg4D in converting LC3-I to LC3-II and two other mediators (STMN1 and RAB5A) also act as regulators for autophagy (Backert et al., 2015).

However, tumor suppressor miRNAs may be autophagy regulators to maintain the function of cytoplasmic proteins or autology cell death, which may play a distinct role in similar biofilms. How does autophagy play the role of Janus in cancer cells? And how miRNAs can act as an oncogene or tumor suppressor in regulating autophagy? To answer these questions, there are two hypotheses: One of the hypotheses is that the role of Janus in autophagy may depend on different stages of cancer, including stem cells, onset, and progression, attack and metastasis, or its latency. For example, autophagy can disrupt the formation of a tumor in the early stages of cancer. While cancer, if formed, is in the interest of cancer invasion and metastasis. Another hypothesis is that excessive expression of oncogenic miRNAs can lead to a reduction in the expression of tumor suppressants by targeting miRNA to tumor formation; in other words, the low expression of tumor suppressor miRNAs can lead to inappropriate expression of miRNAs-targeted oncogenes (Frankel et al., 2011; Ouyang et al., 2012).
In autophagy, oncogenic miRNAs may regulate some of the tumor suppressor signaling pathways (e.g., ULK1 complex, Beclin-1 function, and Atg4 messaging), whereas tumor suppressor miRNAs can affect other oncogenic signaling pathways (e.g., mTORC1 signaling). Interestingly, Beclin-1 is negatively regulated by miRNA-376b (oncogenic miRNA-376b) miRNA and miRNA-30a (tumor suppressor miRNA) as well as Atg4 is inhibited by miRNA-376b (oncogenic miRNA 101 (tumor suppressor) in cancer. So, this apparent paradox can be explained in this way that both Beclin-1 and Atg4 are one oncosuppressor that play different roles in a particular cell or tissue. The genetic and molecular mechanisms involved in the development of gastric cancer are not yet fully understood. Many factors, including bacterial agents, host, and microenvironment can be decisive in the development of the tumor (Tang et al., 2012).

MiRNAs may affect induced autophagy associated with the pathogenesis of *H. pylori* by regulating the transcription and expression of different genes that can play an important role in inflammation, cell proliferation, apoptosis, and differentiation. In fact, it has been reported that *H. pylori* can regulate inflammatory responses by altering the expression of gastric mucosal miRNAs that contribute to the bacterial host responses. MiRNAs play a pivotal role in regulating inflammatory pathways and also carcinogens mediated by inflammation due to *H. pylori* infection. This function may be mediated through DNA methylation and epigenetic changes and suppression of tumor suppressor miRNAs (Jing, Han, Sui, Xie, & Pan, 2015). In addition, *H. pylori*-mediated miRNAs also play a role in regulating the cell cycle, apoptosis, and alteration of epithelial–mesenchyme cells. In general, miRNAs seem to play a key role in the progression of gastritis to neoplastic changes and neoplastic lesions. As each miRNA can control the expression of hundreds or thousands of genes, the recognition of the target genes for these miRNAs and their performances are essential (Z. Zhang et al., 2008).

There is an accepted pattern for the pathogenesis of gastritis and intestinal cancer, which is a multistage progression of *H. pylori* inflammatory/chronic gastritis to gastric atrophy, intestinal metaplasia, dysplasia, and ultimately adenocarcinoma, which was first introduced by Correa. It has been suggested. *H. pylori* are responsible for initiating early stages of gastritis and atrophy and progressing to neoplastic lesions and ultimately gastric cancer but the molecular mechanisms that contribute to the progression of the disease are still not fully understood (Watanabe, Tada, Nagai, Sasaki, & Nakao, 1998). In addition, only a small proportion of people cloning to neoplasm progress, which suggests that viral agents, bacteria, host responses, and environmental factors may affect the risk of cancer (Peek & Blaser, 2002).

In gastrointestinal cancers, some of the miRNAs are low and seem to act as tumor suppressants (e.g., mir-15b and mir-16, which target the antiapoptotic protein Bcl-2, decreases in gastric cancer). In contrast, some miRNAs are expressed in gastrointestinal cancers and act as oncogenes (e.g., mir-155 that suppresses the expression of the proapoptotic protein TP53INP1 and its levels increased in the lymphoma mucosal lymphatic tissue; Jing et al., 2015; Nishizawa & Suzuki, 2013; Wu et al., 2016). *H. pylori* can also modify autophagy through a mechanism that includes the function of miRNAs. Tang et al. found that MIR30B expression increased in the gastric mucosa of infected patients and during the infection an increase of this miRNA was accompanied by an infected gastric epithelial cell line. The expression of modified MIR30B is a reaction to *H. pylori* because the infection with other bacteria does not cause a similar increase. MIR30B directly targets Atg12 and BECN1 to reduce the autophagy and increase the survival of *H. pylori* cells.

## 15 CONCLUSION

*H. pylori* induced defective autophagy or inhibited it by means of CagA and VacA toxins leading to the gastric cancer induction. Impaired or defective autophagy leads to the accumulation of cytotoxic materials, such as ROS and P62 that leads to increased mutations in the DNA and enhance cancer formation. *H. pylori* CagA may inhibit autophagy through the c-Met/PI3k/Akt-mTOR signaling pathway. However, VacA induces autophagy by some signaling pathways. Therefore, it is concluded that VacA is an essential factor for autophagy in gastric epithelial cells that causes the death of autophagic cells through ER stress in gastric epithelial cells. While CagA is a negative regulator of this phenomenon, the elimination of this gene from *H. pylori* has increased autophagy and the production of inflammatory cytokines is reduced. In gastrointestinal cancers, some of the miRNAs act as tumor suppressants and some other are oncogenes by regulating various genes expression. *H. pylori* can also modify autophagy through a mechanism that includes the function of miRNAs. In autophagy, oncogenic miRNAs may regulate some of the tumor suppressor signaling pathways (e.g., ULK1 complex, Beclin-1 function, and Atg4 messaging), whereas tumor suppressor miRNAs can provide other oncogenic signaling pathways (for instance, Beclin-1 is negatively regulated by miRNA-376b (oncogenic miRNA-376b) miRNA and miRNA-30a (tumor suppressor miRNA). Similarly, Atg4 by miRNA-376b oncogenic (miRNA miRNA-101) miRNA-suppressor of the tumor is inhibited in cancer. So, this apparent paradox can be explained in this way that both Beclin-1 and Atg4 played different roles in a particular cell or tissue.

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## REFERENCES


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