Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Review

Cellular evasion strategies of *Helicobacter pylori* in regulating its intracellular fate

Wei Yang Sit,b,1, Yu-An Chen,c,d,1, Yu-Lun Chen,a,b, Chih-Ho Lai,d,e,f,g,⁎, Wen-Ching Wang,a,b,*

a Biomedical Science and Engineering Center, National Tsing Hua University, Hsinchu, Taiwan
b Institute of Molecular and Cellular Biology & Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan
c Department of Urology, University of Texas Southwestern Medical Center, Dallas, TX, 75390, USA
d Department of Microbiology and Immunology, Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan
e Department of Microbiology, School of Medicine, China Medical University, Taichung, Taiwan
f Department of Nursing, Asia University, Taichung, Taiwan
g Molecular Infectious Disease Research Center, Department of Pediatrics, Chang Gung Memorial Hospital, Linkuo, Taiwan

ARTICLE INFO

Keywords:
*Helicobacter pylori*
Autophagy
Virulence factor
Intracellular survival

ABSTRACT

*Helicobacter pylori* colonizes human stomach mucosa and its infection causes gastrointestinal diseases with variable severity. Bacterial infection stimulates autophagy, which is a part of innate immunity used to eliminate intracellular pathogens. Several intracellular bacteria have evolved multipronged strategies to circumvent this conserved system and thereby enhance their chance of intracellular survival. Nonetheless, studies on *H. pylori* have produced inconsistent results, showing either elevated or reduced clearance efficiency of intracellular bacteria through autophagy. In this review, we summarize recent studies on the mechanisms involved in autophagy induced by *H. pylori* and the fate of intracellular bacteria.

1. Introduction

The immune system of healthy individuals comprises potent mechanisms to protect the body against microbial infections. Autophagy is an evolutionarily conserved process to eliminate pathogens [1]. This catabolic process sequesters cytosolic components including misfolded proteins, injured organelles, and intracellular pathogens through a lysosome-dependent pathway [2]. Accumulated evidence shows that successful pathogens can hijack autophagy for their own replication [3]. This review focuses on a chronic gastric pathogen, *Helicobacter pylori*, which often inhabits mucosal layers and causes dyspeptic symptoms of varying severity. Findings of previous studies indicate that autophagy plays an important role in host defense, lessening *H. pylori* burden [4–7]. However, *H. pylori* is a facultative intracellular pathogen and is capable of invading and surviving not only in non-phagocytic cells but also in professional phagocytes [8–11]. In addition, *H. pylori* is detected in double-layered autophagosomes, indicating that autophagosomes serve as a peculiar niche for *H. pylori* multiplication to sustain intracellular infections [8,12,13]. However, the underlying mechanisms through which *H. pylori* coopts the autophagy machinery to affect bacterial clearance efficiency in host cells remain to be elucidated.

2. *H. pylori* virulence factors

2.1. Adhesion molecules

Microorganisms use bacterial adhesins to bind to host cells, protecting themselves from mechanical attack, such as acidic pH and mucosal liquids [14]. Genomic analysis reveals that *H. pylori* contains more than 30 outer membrane protein (omp) genes including the *Helicobacter OMPs* (Hop) and the *hop*-related group (*hor*) [15]. The two most important adhesins in *H. pylori* are Lewis b (Leb) blood group antigen-binding adhesin (BabA) and sialyl Lewis X antigen-binding adhesin (SabA), and both these adhesins are Hop proteins [16–18]. Thus, the host and pathogen interact partly through the binding of adhesins to specific carbohydrate moieties of the gastric epithelium, promoting infection and inflammatory processes in the gastrointestinal tract.
2.2. Vacuolating cytotoxin A (VacA)

VacA, which causes massive vacuolation in cultured cells, was first observed in culture supernatants of toxic *H. pylori* isolates [19]. Purification and subsequent studies on this unique toxin showed that VacA is synthesized as a 140-kDa polypeptide precursor, which undergoes trimming to yield a mature 95-kDa toxin and is secreted via an autotransporter encoded in its C-terminal domain [20–22]. It can assemble into water-soluble oligomeric forms including a single layer structure consisting of hexamers/heptamers and a double-layered assembly [23]. It is interesting that VacA disassembles at acidic pH, interacts with the lipid membrane and reassembles into a pore-forming hexameric structure in membranes, enabling the transport of chloride ions [22,24]. This unique channel-formation activity contributes to vacuolation in late endosomal and lysosomal compartments as well as increases in mitochondrial membrane permeability which leads to cytochrome c release [22,23].

Several surface molecules have been reported as VacA receptors. Receptor-like protein tyrosine phosphatase β (RPTPβ) binds to VacA through its terminal sialic acid domain in gastric epithelial cells [25,26]. RPTPα also serves as a functional VacA receptor in human kidney tumor cell G401 that lacks RPTPβ [27]. The interaction of VacA and RPTPα activates Src phosphorylation, resulting in CagA phosphorylation at Tyr972 in AZ-521 cells [28]. Notably, VacA is able to target the membrane of multiple cell types including gastric epithelial cells, T cells, and parietal cells. Therefore, VacA with high pore-forming activity is associated with increased disease severity [29]. It has been described that removing the membrane-associated region of VacA blocks its vacuolating activity and prevents VacA from integrating into the inner-mitochondrial membrane [30]. Recent evidence also shows that VacA binds to low-density lipoprotein receptor-related protein 1 (LRP1) and is involved in triggering autophagy as discussed below [31].

2.3. CagA and type IV secretion system (T4SS)

CagA is the gene product encoded within the 40-kb *cag*-pathogenicity island (*cag*-PAI) which also includes 31 additional genes that encode the T4SS [32]. The mechanisms that pathogens have exploited in order to introduce the virulence factors into the host cells. In another study conducted, *H. pylori* cag-T4SS interacts with the β1 integrin of the host cells in an arginine-glycine-aspartate (RGD)-independent manner, which initiates effector protein translocation [33]. When *H. pylori* contacts with the host membrane, CagA interacts with the externalized phosphatidylycerine to facilitate its entry into target cells, which is important for the pathophysiological activity of CagA [34]. In addition to β1 integrin, a recent study by König et al. describes that HopQ specifically interacts with human carcioembryonic antigen-related cell adhesion molecule family (CEACAMs), facilitating CagA translocation into host cells [35]. These studies suggest that *H. pylori* exploits multiple ways to exert CagA translocation into the host cells. The translocated CagA can be phosphorylated and interacted with several host signaling proteins, thus interfering with host cell signaling [36]. It is known that CagA translocation and phosphorylation are mediated through cholesterol-rich microdomains present on the cytoplasm membrane (also referred as lipid rafts) [34,37–39]. Lipid rafts are important for efficient T4SS-mediated CagA translocation, inducing an elevated level of the cell hummingbird phenotype and increased IL-8 production [37]. The host membrane lipid phosphatidylycerine plays a crucial role in CagA delivery and localization [34,40]. Moreover, simvastatin, a HMG-CoA reductase inhibitor, which reduces the level of cellular cholesterol and decreases translocation and phosphorylation of CagA, indicating that cholesterol is crucial for CagA-mediated actions [39].

2.4. Cholesterol-α-glucosyltransferase (CGT)

The O-Glycans of the human gastric mucosa diminish the levels of *H. pylori* infection by inhibiting the bacterial cholesterol-modifying enzyme, cholesterol-α-glucosyltransferase (CGT) encoded by the type 1 capsular polysaccharide biosynthesis protein J (*capJ*) [41]. CGT catalyzes the conversion of host cholesterol into cholesterol-α-glucoside (aCG) [41]. Further modification of aCG yields cholesterol-6′-O-acetyl-a-glucoside, cholesterol-6′-O-phosphatidyl-a-glucoside, and cholesterol-6′-O-lysophosphatidyl-a-glucoside, which constitute a large proportion of the glycolipids in the cell wall [41]. Cholesterol glucosylation of *H. pylori* by CGT, interestingly, promotes *H. pylori* evasion of immune defenses [42]. We have previously shown that knockout of *capJ* (∆CapJ), which abolishes the production of cholesterol glucosides (CGs), greatly decreases the degree of T4SS-induced activities, including CagA translocation/phosphorylation, subsequent signaling events, the scattering hummingbird morphology in AGS cells, and IL-8 secretion [43]. This is mainly because CGs of the wild-type *H. pylori*-infected AGS cells are essential for reorganizing the lipid-raft membranes for efficient T4SS function [43]. Furthermore, cholesterol-glycosylated *H. pylori* is largely internalized via a lipid-raft-dependent pathway, which delays the maturation of phagosomes and facilitates the evasion of macrophages [44], suggesting a role in interfering with autophagy, which is discussed in a later section [45]. *H. pylori* harboring CGT, inhibits interferon gamma-induced signaling, which avoids host inflammatory responses [46]. Noticeably, CGs also contribute to cell wall integrity, morphology, and antibiotic resistance [47]. Collectively, these findings highlight the multiple strategies employed by *H. pylori* for persistent colonization.

2.5. Other virulence factors

Other virulence factors are also engaged in *H. pylori*-induced pathogenesis, such as urease, flagella, and heat-shock proteins (Hsp). Infection of *H. pylori* upholds the periplasmic pH to adapt to the acidic environment of stomach by means of urease [48]. The increased levels of ammonia from hydrolysis of urea by as well as the metabolites from recruited neutrophils disrupts the gastric epithelium, which synergistically facilitates the development of gastric malignancies [49,50]. In addition, *H. pylori* possesses four to eight unipolar flagella that are important in bacterial colonization and induce inflammatory response [51]. The flagellar sheath of *H. pylori* provides the protection of acid-labile flagellar structure from the attack of the acid environment [52]. Additionally, *H. pylori* harbors two main heat-shock proteins, GroES-like HspA (Hsp10), and GroEL-like HspB (Hsp60). The regulation of heat-shock proteins expression by *H. pylori* is crucial for bacteria to adapt and survive in the hostile environment of the stomach [53].

3. Autophagy in bacterial infection and pathogenesis

3.1. Composition, structure, and functions of autophagy

Autophagy is an intracellular degradation process by which cytoplasmic constituents are degraded by the lysosome [54]. The three major forms of autophagy include microautophagy, macroautophagy, and chaperone-mediated autophagy [55,56]. Microautophagy and chaperone-mediated autophagy are directly recruited to a lysosome, which engulfs small portions of cytosol and receives chaperone-associated cargo [57]. Macroautophagy can sequester cytosolic molecules, including damaged organelles or pathogens, in the autophagosome [57]. In mammalian cells, macroautophagy occurs in the cytoplasm; the organelles are surrounded by a phagophore or cytoplasmic double-membrane-bound structure, which start growing at both ends to create an autophagosome [58,59]. The autophagosome subsequently fuses with a lysosome, forming a single, large, and membrane-surrounded vesicle (autophagolysosome); both the membrane and its contents are
then degraded by lytic enzymes [60,61]. Autophagy has been demonstrated to play crucial roles in biological processes, such as cell protection, response to starvation, recycling nutrients from digested organelles and macromolecules by removing damaged organelles and aberrantly folded proteins, diseases, and host defenses [62].

### 3.2. Bacterial infection and the autophagy pathway

The large-scale degradation of invading pathogens by autophagy, called xenophagy, has been commonly used to describe the host defense response to effectively eliminate the invading pathogens including intracellular bacteria [63]. Various studies have been conducted on different pathogens and how their virulence factors manipulate the autophagy pathways of their hosts to promote their intracellular survival and multiplication. Examples of such pathogens include Mycobacterium tuberculosis, Streptococcus pyogenes, Shigella flexneri, Salmonella typhimurium, Listeria monocytogenes, and Legionella pneumophila, all of which have been extensively studied to elucidate the strategies they use to alter the host autophagy pathway (Table 1). The details of the underlying mechanism will be covered later.

### 3.3. Adhesion and invasion of cells

Pathogenic microbes exhibit numerous approaches for evading immune defenses and developing a protected niche within the host. Therefore, the interplay between the initial entry of these pathogenic microbes into the host cells and the response of the host cells plays a critical role in determining the fate of the microbes. Wang and Hajishengallis previously reported that Porphyromonas gingivalis relies on the lipid rafts of the macrophages to enhance its intracellular bacterial load and survival [64]. There is mounting evidence that shows parasites [65–67], viruses [68,69], and prions [70,71] target the lipid rafts of the host cells, which benefits infections. Lipids cluster together to form microdomains in cell membranes, i.e., lipid rafts. Lipid rafts composed of high concentrations of cholesterol and glycosphingolipids [72], that modulate a wide array of cellular processes such as signal transduction [73], membrane trafficking [74,75], and cytoskeletal organization [76,77].

Various studies have been shown that lipid rafts are common platforms for bacteria to adhere and invade to host cells [78]. A study conducted by Shin et al. demonstrated that lipid rafts serve as important docking points for bacteria that express FimH [79]. Furthermore, these bacteria reside in vacuoles containing the lipid rafts, which is important for promoting their intracellular survival. H. pylori also exploits lipid rafts for initial adhesion and invasion. A study conducted by Wunder et al. demonstrated that H. pylori is auxotrophic for cholesterol and is able to extract cholesterol from the lipid rafts of the host, resulting in immune evasion [42]. This study also corroborates our previous findings that lipid rafts of the epithelial cells play a key role in H. pylori internalization [37]. In addition to bacteria, viruses also rely on lipid rafts for attachment and invasion. The coronavirus, infectious bronchitis virus is reliant on the host’s lipid rafts for attachment and thereby promoting viral infection [80].

### 3.4. Cytoplasmic trafficking

Upon invasion of host cells by the pathogens, they can modulate the host cell trafficking machinery to promote evasion from the host innate immune response through Rab GTPases which are proteins responsible for vesicular trafficking, at least in part [81]. Rab proteins, when activated, interact with a large number of Rab effector proteins to regulate different cellular functions such as membrane trafficking and intracellular signaling [82]. Rab proteins reside in intracellular membrane compartments of the plasma membrane, mitochondria, and the nucleus and are involved in a large number of cellular functions [83]. Above all, Rab proteins are known for their roles in the endocytic/exocytic pathways, the retrograde/anterograde secretory pathways, and membrane recycling [84].

As xenophagy involves the trafficking of pathogens in the host cells to the lysosomes for degradation, various studies have demonstrated that different pathogens target different Rab proteins to promote their intracellular survival. For instance, M. tuberculosis modifies Rab22, which is associated with the maturation of phagosomes [85], and promotes the accumulation of Rab14 in M. tuberculosis containing phagosomes, preventing fusion of the phagosome with lysosome [86].

While Rab proteins serve as one of the key targets for pathogens, other molecules are also triggered by pathogens to manipulate the host’s cytoplasmic trafficking. Salmonella secretes secretor effector protein (SifA) via its type 3 secretion system (T3SS) from the Salmonella-containing vacuoles to the cytoplasm [87]. SifA then binds to kinesin-interacting protein (SKIP), a host protein, which down-regulates the recruitment of kinesin to the bacterial vacuoles, thereby promoting the membrane dynamics of vacuoles [88]. Upon invading the host cells, a few pathogens escape from the vacuoles and replicate in the host cytoplasm. These pathogens express factors that initiate the process of actin polymerization, facilitating their propagation in the cells. For example, L. monocytogenes secretes the virulence factor listeriolysin O (Llo), a pore-forming exotoxin which degrades the membrane of internalized vacuoles in the infected host cells [89], eventually resulting in vacuolar dissolution, and thereby promoting intracellular survival [90]. Furthermore, the pathogen is able to translocate across the infected cell wall to spread and invade adjacent cells through actin-based motility [91]. This phenomenon is attributable to the presence of the protein actin assembly-inducing protein (ActA) on the bacterial surface. ActA acts as a nucleator, which induces actin polymerization allowing the bacteria to move forward in the host cells [92].

### 3.5. Bacterial survival or elimination in host cells

Upon entering host cells, several pathogens evade phagosome fusion with the degradative lysosome through different mechanisms thereby increasing their survival and bacterial load in the host cells. One of the mechanisms by which pathogens promote their intracellular survival is by arresting phagosome maturation. To achieve this, pathogens are capable of obstructing phagosomal lipid metabolism. One example is M. tuberculosis, which produces a phosphatase identified as secreted acid phosphatase (SapM). SapM is capable of converting PI(3)P to PI, inhibiting late phagosome endosome fusion which in turn inhibits phagosomal maturation [93]. Another example is Salmonella enterica,
is subsequently recognized by the autophagic machinery but delays phagocytosis by using its effector protein RavZ. In the autophagosome, L. pneumophila also delays the fusion of the autophagosome with lysosome to allow it to time to develop into an acid-resistant form. This acid-resistant form of L. pneumophila can then replicate in the acidic autophagolysosome.

which injects effector sigma D factor (SigD) into host cells, leading to the modulation of phosphatidylinositol 3-kinase (PI3K) and increasing vacuolar PI(3)P, thereby avert phagosome maturation and preventing fusion with a lysosome [94].

A second method used by pathogens to evade host cell degradation is escaping from the host cell phagosome. Studies on Mycobacteria species have shown that they activate host cytosolic phospholipase A2 to assist the microbe in escaping the phagosome [95]. This clearly demonstrates the highly adaptable strategy of Mycobacteria for surviving in host cells (Fig. 1). Recently, another survival strategy employed by the pathogen has been discovered. While employing this strategy, the pathogen directly interferes with the maturation of the autolysosome, circumventing autophagic degradation [96].

In contrast to the abovementioned examples where pathogens subvert autophagy to promote their intracellular survival, some pathogens that are eliminated by activating autophagy. Gutierrez et al. demonstrated that activation of autophagy by starvation or treatment with rapamycin triggers an effective clearance of M. tuberculosis in macrophages [97]. Similarly, using rapamycin to activate autophagy led to an enhancement of targeting Burkholderia cepacia to lysosomal compartments, resulting in better prognosis in patients with cystic fibrosis [98]. Notably, a new LC3-associated phagocytosis (LAP) autophagic machinery has been recently identified [99]. The key function of LAP is to enhance fusion of phagosomes and lysosomes, which promotes pathogen killing [100]. However, the detailed mechanism by which LAP enhances phago-lysosome fusion remains to be studied.

4. H. pylori modulates autophagy

Autophagy as an intracellular defense to eliminate pathogens can be induced by H. pylori in both gastric epithelial cells [13,96,101,102] and professional phagocytes [103,104]. Recently accumulating evidence has shown that H. pylori exploits host autophagy to survive within cells and even multiply [10,105,106]. However, the mechanisms vary depending on the host cells or bacterial strains. In this section, we summarize recent studies conducted to elucidate the different mechanisms of autophagy of H. pylori in gastric epithelial cells and professional phagocytes.

4.1. H. pylori infects gastric epithelial cells

Several virulence factors and host genes have been shown to affect H. pylori-induced autophagy (Table 2). Of these, VacA was first reported by Terebiznik et al. to induce autophagy in the AGS cell line [101]. H. pylori infection of AGS cells increases LC3 puncta and promotes the conversion of LC3-I to LC3-II. Moreover, formation of LC3 puncta was decreased in atg5−/− mouse embryonic fibroblasts (MEFs) challenged with H. pylori, indicating that H. pylori-induced autophagy in AGS cells follows the canonical pathway [101]. Treatment of AGS cells with purified toxin or conditioned culture media supernatants revealed that VacA but not CagA, CagE, urease, or the TSSS is essential and sufficient to induce autophagy [101]. In contrast, another study using AZ-521 cells found that treatment with 3-methyladenine (3-MA), a PI3K inhibitor, or knockdown of BECN1 did not inhibit VacA-induced autophagy, suggesting that it does not use the canonical pathway [31]. This finding contradicts the results in AGS cells reported by Tang et al. [102], possibly due to the different cell lines used. Interestingly, autophagy in turns modulates the activity of intracellular VacA, indicating that autophagy protects host cells against H. pylori infection. However, the mechanism by which these virulence factors induce autophagy remains to be investigated. One clue is that VacA damages mitochondrdial function and causes nutrient depletion which may be linked to inhibition of mTOR signaling and induction of autophagy [107].

Many studies have reported that H. pylori can prevent its degradation, allowing it to reside and multiply in autophagosomes. Raju et al. showed that prolonged exposure (24 h) to a toxin disrupts subsequent autophagosome-lysosome fusion and causes the accumulation of autophagosomes in the cells [108]. This observation suggests that the underlying mechanisms of autophagy are not the same in acute and chronic exposure (Fig. 2). The final step of autophagy is formation of autolysosomes, which are the fusion of autophagosomes and lysosomes. For instance, after prolonged exposure to conditioned culture media, AGS cells show a reduced level of the lysosomal protease cathepsin D,
**Table 2**

*H. pylori* manipulates autophagy in phagocytic and non-phagocytic cells.

| Bacterial virulence factors (V) or host determinants (H) | Factor | Cell line used | Role in intracellular survival and/or autophagic processes | Reference |
|----------------------------------------------------------|--------|----------------|----------------------------------------------------------|-----------|
| **Epithelial cells**                                       |        |                |                                                         |           |
| **V**                                                     | CagA   | AGS            | Does not involve in *H. pylori*-induced autophagy         | [101]     |
|                                                          |        |                | Promote *H. pylori* intracellular survival                 | [13]      |
|                                                          | CagE   | AGS            | Does not involve in *H. pylori*-induced autophagy         | [101]     |
|                                                          | VacA   | AGS            | VacA channel-forming activity is essential for the induction of autophagy | [101]     |
|                                                          |        | A2521          | Induce autophagy by binding to LRP1                       | [31]      |
|                                                          |        | AGS            | Prolong exposure may cause autophagy disruption due to lack of cathepsin D in lysosome | [107]     |
|                                                          | BabA   | AGS            | Inhibit *H. pylori* internalization and invasion           | [13]      |
|                                                          | Urease | AGS            | Does not involve in *H. pylori*-induced autophagy         | [101]     |
|                                                          | ATG5   | AGS            | Essential for *H. pylori*-induced autophagy               | [101]     |
|                                                          | ATG12  | AGS            | Essential for *H. pylori*-induced autophagy               | [101]     |
|                                                          | ATG16L1| AGS            | Single nucleotide polymorphism ATG16L1300A may increase the intracellular survival of *H. pylori* | [107]     |
|                                                          | BECN1  | AGS            | Essential for *H. pylori*-induced autophagy               | [102]     |
|                                                          |        | A2521          | Does not involve in VacA+-*H. pylori* autophagy           | [31]      |
|                                                          |        | HFE145         | Promote *H. pylori* intracellular survival by inhibiting autophagosome formation | [96]      |
|                                                          |        | LRP1           | Induce autophagy by binding to VacA                        | [31]      |
|                                                          |        | MIR30B         | Essential for *H. pylori*-induced autophagy by downregulating ATG12 and BECN1 | [102]     |
|                                                          |        | MIR30D         | Essential for *H. pylori*-induced autophagy by downregulating genes involved in autophagy pathway | [112]     |
|                                                          |        | PtdIns3K       | Does not involve in *H. pylori*-induced autophagy         | [31]      |
| **H**                                                     |        |                |                                                         |           |
|                                                          |        | ATG16L1        | Single nucleotide polymorphism ATG16L1300A may increase the intracellular survival of *H. pylori* | [107]     |
|                                                          |        | ATG16L1        | Essential for *H. pylori*-induced autophagy               | [102]     |
|                                                          |        | ATG16L1        | Single nucleotide polymorphism ATG16L1300A may increase the intracellular survival of *H. pylori* | [107]     |
| **Professional phagocytes**                               |        |                |                                                         |           |
| **V**                                                     | CagA   | THP-1          | Inhibit *H. pylori* internalization and intracellular survival | [103]     |
|                                                          |        | BMDCs          | Promote *H. pylori* intracellular survival                 | [104]     |
|                                                          | VacA   | THP-1          | Promote *H. pylori* intracellular survival                 | [103]     |
|                                                          | BMDCs  | BMDCs          | Promote *H. pylori* intracellular survival                 | [104]     |
|                                                          | CapJ   | J774A.1        | Delay the fusion of autophagosome and lysosome             | [45]      |
|                                                          |        | J774A.1        | Delay *H. pylori* internalization                          | [44]      |
| **H**                                                     | ATG16L1| PMBCs          | Single nucleotide polymorphism ATG16L1300A may increase the intracellular survival of *H. pylori* | [107]     |
|                                                          |        | PtdIns3K       | Inhibit the bacterial multiplication                      | [103]     |
|                                                          |        | RAW264.7       | Does not inhibit the bacterial multiplication             | [103]     |

Fig. 2. *H. pylori* modulates autophagy in epithelial cells. VacA is an essential virulence factor for modulating *H. pylori* induced autophagy via various mechanisms. The initiation of infection starts with the internalization of *H. pylori* and VacA, the formation of VacA pores or the binding of VacA to LRP1. The internalization of *H. pylori* and VacA may induce autophagy. VacA forms pores in the mitochondria, causing nutrient depletion which induces autophagy, mediated through the inhibition of mammalian target of rapamycin complex 1 (mTORC1). Autophagy may proceed through either the canonical or non-canonical pathway depending on the cell lines used. In addition to inducing autophagy, *H. pylori* can hijack the autophagy process. *H. pylori* infection may induce the expression of MIR30B and MIR30D, which subsequently inhibit autophagy by targeting different autophagy-related genes. Also, the acidification of lysosome is disrupted by chronic exposure to VacA, causing the accumulation of pro-cathepsin D. These mechanisms allow *H. pylori* to survive and replicate in the autophagosome.
due to disruption of lysosome acidification [108]. The defective autophagosomes in AGS cells caused by VacA may lead to the accumulation of SQSTM1, a major cargo ubiquitin-binding receptor. This phenomenon is also observed in patient biopsies [108]. Other reports also showed the accumulation of different proteins due to VacA-disrupted autophagy, leading to changes in cellular ROS levels [109] and gene mutations [110]. The accumulation of p62 activates nuclear factor erythroid 2-related factor (NRF2) which controls the expression of ROS detoxification genes in AGS cells [108,110]. A contradictory result showed that CagA and HspB, but not VacA, regulate the antioxidant response [111]. Therefore, a detailed investigation of the effect of p62 and NRF2 on *H. pylori* virulence factors that regulate autophagy is warranted.

Consistent with the previous findings, a recent study has also shown reduced activity of lysosomal proteases including cathepsin D, N-acetyl-β-D-glucosaminidase (β-NAG), and acid phosphatase in HFE-145 cells infected by *H. pylori* [96]. Disruption of lysosome acidification causes the repression of the retrograde trafficking of mannose-6-phosphate receptors which play an important role in carrying lysosomal hydrolytic enzymes in the trans-Golgi network [96]. However, the mechanism underlying the disruption of lysosome acidification in *H. pylori* infected cells is still unclear.

The internalization of VacA or *H. pylori* plays a critical role at the beginning of autophagy. There is evidence that VacA induces autophagy by binding to LRPs [31]. Interestingly, s1m1 VacA, but not s1m2 or s2m2 interacts with LRPs and stimulates autophagy [31]. Moreover, knockdown of LRPs significantly reduces autophagy, indicating that VacA internalization is crucial for the induction of autophagy. Internalized VacA may further alter the membrane permeability of mitochondria, causing the release of cytochrome c and leading to the induction of mitophagy and apoptosis [29,111]. In addition, the internalization of *H. pylori* is unaffected by VacA and CagA, but is correlated with *H. pylori* invasin NudA in AGS cells [112]. Notably, the internalization of *H. pylori* also requires the activation of PI3-kinase and PKC in AGS cells [105].

In addition, although VacA induces autophagy, *H. pylori* may evade autophagy process by altering the expression of various microRNAs, including MIR30B and MIR30D [102,112]. *H. pylori* infection may increase the expression of MIR30B in different gastric cell lines (i.e. MIR30D in AGS and GES1 cells). Both MIR30B and MIR30D target several genes involved in autophagy, such as ATG12 and BECN1. Interestingly, a very recent report by Tsugawa et al. showed that overexpression of capping actin protein of muscle Z-line alpha subunit 1 (CAPZA1) blocks the CagA-degraded autophagy by downregulating the expression of lysosomal associated membrane protein 1 (LAMP1) in *H. pylori*-infected cells [113]. Moreover, Tsugawa et al. showed that CagA accumulation in CAPZA1-overexpressing cells is essential for the up-regulation of CD44v9 which is a cancer stem-cell marker, suggesting that overexpression of CAPZA1 could be a key risk factor in gastric cancer development [114]. This notion is supported by an earlier study that the abundance of CD44v9 is a superior prognostic predictor than the presence of open-type gastric mucosal atrophy for the recurrence of gastric cancer in patients after the resection of early gastric cancer [115]. These lines of evidence together suggest that *H. pylori*-infected gastric mucosa exhibits negative regulation of autophagy through an orchestrated array of actions from multiple virulence factors. As such, *H. pylori*-mediated defective autophagy links to an increased gastric cancer risk, thereby promoting the development of various gastrointestinal diseases.

### 4.2. *H. pylori* manipulates autophagy in professional phagocytes

Compared to gastric epithelial cells, the effects of *H. pylori*-induced autophagy by professional phagocytes are much more complicated due to differences between the cell lines and bacterial strains used. The primary role of phagocytes is to ensure the efficient clearance of harmful foreign particles, bacteria, and dead or dying cells. In the case of *H. pylori* infection, various studies have demonstrated its capability to delay phagocytosis by phagocytes.

Wang *et al.* first reported that *H. pylori* induces activation of autophagy in macrophages [103]. In another previous study, *H. pylori* infection was found to increase the number of LC3-puncta in both RAW264.7 and THP-1 cells [12]. In addition to activation of autophagy, Wang *et al.* further demonstrated that *H. pylori* multiplies in human monocyte THP-1 or U937 cells, but not in murine macrophage RAW264.7 cells. Another report showed that *H. pylori* could replicate in the autophagosomes of mouse bone marrow-derived dendritic cells (BMDCs) [104]. Moreover, *H. pylori* infection of BMDCs may impair MHC class II molecule surface expression which is negatively regulated by Toll-like receptor (TLR)-2 and 4. The correlation of TLR4 polymorphism and gastric cancer may deserve further attention [116]. Interestingly, both studies show that CagA and VacA are important for the survival and multiplication of *H. pylori* in autophagosomes [103,104]. These results contradicted the observations in gastric epithelial cells, which may be due to the different types of cell lines and *H. pylori* strains used.

This observation corroborates with another study where pre-loading *H. pylori* with cholesterol enhances the rate of *H. pylori* internalization [117]. We recently have shown that CGT, a main factor in *H. pylori* cholesterol glucosylation, promotes autophagosome formation, and simultaneously inhibits lysosome formation in murine macrophages to improve its intracellular survival [45]. This study also demonstrated that ATG12 is involved in the autophagy pathway, which is essential for *H. pylori* intracellular survival [45]. In addition, treatment of macrophages with siRNAs targeting autophagy and thereby reduced intracellular bacterial burden [7] (Fig. 3).

*H. pylori* induced autophagy in phagocytes is affected by the host genotype. For instance, several gene polymorphisms are associated with Crohn disease, an inflammatory bowel disorder, which affects *H. pylori* induced autophagy. ATG16L1, which is involved in autophagosome formation with the T300A mutation makes host more susceptible to *H. pylori* infection and reduces *H. pylori*-induced autophagy [108]. However, the detailed underlying mechanism remains to be explored. Another gene involved in this autophagy is the nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a pattern recognition receptor. During *H. pylori* infection, NOD2 activates nuclear factor-kappa B (NF-kB) signaling which induces inflammation. However, the R702W mutant cannot activate NF-kB signaling [118]. Therefore, the mutation is associated with gastric MALT lymphoma, which is usually associated with *H. pylori* infection [118]. In addition to NOD2, TLR4, a pattern recognition receptor, also plays an important role in stimulating macrophage autophagy by binding to lipopolysaccharides on the bacteria [7,45]. TLR4 polymorphisms have been shown to correlate with gastric cancer [116]. However, further experiments are needed to reveal the underlying mechanisms.

### 5. Conclusions and perspectives

Autophagy is increasingly recognized as a vital route for host defense. Thus, it is not surprising that successful intracellular pathogens are evolved to dysregulate autophagy, contributing to disease pathogenesis. *H. pylori*, a small pathogen, shows remarkable capabilities to enhance its intracellular survival and propagation by means of a limited number of virulence factors, exemplified by multiple strategies that have evolved to negatively regulate autophagy. This, in turn, increases the risk of developing *H. pylori*-associated diseases. However, there are discrepancies in the results obtained to date, mainly due to the different types of cell models and/or bacterial strains used. Furthermore, the connection between virulence factors, dysregulated intracellular trafficking, disease etiology and its clinical relevance remains largely unanswered. For instance, does CagA, VacA, or CGT mediate membrane remodeling and interfere with intracellular trafficking, and how does
Another study has shown that phages by clustering lipid rafts. The disrupted lipid rafts result in a lower internalization of autophagy. Recent studies have shown that HP0421, a cholesterol-α-glucosyltransferase (CGT), modulates autophagy and promotes bacterial survival in macrophages by clustering lipid rafts. The disrupted lipid rafts result in a lower internalization of autophagy. Another study has shown that \textit{H. pylori} infection may increase the expression of major histocompatibility complex II (MHC II). However, TLR4 plays a negative role on BMDC maturation by inhibiting this process.

**Declaration of Competing Interest**

The authors did not have conflicts of interest to declare for this work.

**Acknowledgments**

The authors would like to thank the editors and reviewers for the editorial assistance and their valuable comments. This study is supported by Taiwan Ministry of Science and Technology (MOST108-2811-B-007-507, 108-2321-B-007-004, 107-2627-M-007-004, and 106-2320-B-182-012-MY3), Chang Gung Memorial Hospital (CMRPD10061-3, CMRPD10021-3, and BMRPE90), and Tomorrow Medical Foundation.

**References**

[1] T. Shintani, D.J. Klionsky, Autophagy in health and disease: a double-edged sword, Science 306 (5698) (2004) 990–995.
[2] S. Siqueira Mina, M. Ribeiro Ribe, L.H. Travassos, Autophagy and its interaction with intracellular bacterial pathogens, Front. Immunol. 9 (2018).
[3] V. Deretic, B. Levine, Autophagy, immunity, and microbial adaptations, Cell Host Microbe 5 (6) (2009) 527–540.
[4] N. Mizushima, B. Levine, A.M. Cuervo, D.J. Klionsky, Autophagy fights disease through cellular self-digestion, Nature 451 (7182) (2008) 1069–1075.
[5] Z. Zhao, B. Fux, M. Goodwin, J.R. Dunay, D. Strong, B.C. Miller, et al., Autophagosome-independent essential function for the autophagy protein Atg5 in cellular immunity to intracellular pathogens, Cell Host Microbe 4 (5) (2008) 458–469.
[6] J.C. Yang, C.T. Chien, A new approach for the prevention and treatment of \textit{Helicobacter pylori} infection via upregulation of autophagy and downregulation of apoptosis, Autophagy 5 (3) (2009) 413–414.
[7] W.C. Liao, M.Z. Huang, M.L. Wang, C.J. Lin, T.L. Lu, H.R. Lo, et al., Statin decreases \textit{Helicobacter pylori} burden in macrophages by promoting autophagy, Front. Cell. Infect. Microbiol. 6 (2017) 203.
[8] Y.H. Wang, J.J. Wu, H.Y. Lei, When \textit{Helicobacter pylori} invades and replicates in the cells, Autophagy 5 (4) (2009) 540–542.
[9] A.M. Petersen, K.A. Kroegel, \textit{Helicobacter pylori}: an invading microorganism? A review, FEMS Immunol. Med. Microbiol. 36 (3) (2003) 117–126.
[10] A. Dubois, T. Boren, \textit{Helicobacter pylori} is invasive and it may be a facultative intracellular organism, Cell. Microbiol. 9 (5) (2007) 1108–1116.
[11] C.H. Lai, C.H. Kuo, F.Y. Chen, S.K. Poon, C.S. Chang, W.C. Wang, Association of antibiotic resistance and higher internalization activity in resistant \textit{Helicobacter pylori} isolates, J. Antimicrob. Chemother. 57 (3) (2006) 466–475.
[12] N.S. Deen, L. Gong, T. Naderer, R.J. Devenish, T. Kwok, Analysis of the relative membrane protein expression profile of \textit{Helicobacter pylori} by retagging, Science 279 (5349) (1998) 373–377.
[13] J. Mahdavi, B. Sonden, M. Hurtig, F.O. Olfat, L. Forsberg, N. Roche, et al., \textit{Helicobacter pylori} SabA adhesin in persistent infection and chronic inflammation, Science 299 (5581) (2002) 573–578.
[14] B. Peck, M. Örtkamp, K.D. Diehl, E. Hundt, B. Knapp, Conservation, localization and expression of HopZ, a protein involved in adhesion of \textit{Helicobacter pylori}, Nucleic Acids Res. 27 (16) (1999) 3325–3333.
[15] R.D. Leunk, P.T. Johnson, B.C. David, W.G. Kraft, D.R. Morgan, Cytotoxic activity in broth-culture filtrates of \textit{Campylobacter pylori}, J. Med. Microbiol. 26 (2) (1988) 93–99.
[16] T.L. Cover, M.J. Blaser, Purification and characterization of the vacuolating toxin from \textit{Helicobacter pylori}, J. Biol. Chem. 267 (15) (1992) 10570–10575.
[17] X. Wang, R. Wattiez, C. Pagliaccia, J.L. Telford, J. Ryskaichert, V. Gabiaux, Membrane topology of VacA cytotoxin from \textit{H. Pylori}, FEBS Lett. 481 (2) (2000) 96–100.
C. Athmann, N. Zeng, T. Kang, E.A. Marcus, D.R. Scott, M. Retschkos, et al., Local pH elevation mediated by the intrabacterial urease of Helicobacter pylori, Cell. Microbiol. 28 (3) (2006) 2776–2777.

C. Wunder, Y. Churin, F. Winaw, D. Warnecke, M. Vieth, B. Lindner, et al., Cholesterol glucosylation promotes immune evasion by Helicobacter pylori, Nat. Med. 12 (9) (2006) 1030–1038.

H.J. Wang, W.C. Chen, H.H. Cheng, C.H. Lai, W.C. Wang, Helicobacter pylori cholesterol glucosyltransferase interacts with host membrane phase and affects type IV secretion system function during infection in A549 cells, Microbiol. Mol. Biol. 81 (1) (2012) 67–84.

S.Y. Du, H.J. Wang, H.H. Cheng, S.D. Chen, L.H.C. Wang, W.C. Wang, Cholesterol glucosylation by Helicobacter pylori delays internalization and arrests phagosome maturation in macrophages, J. Microbiol. Immunol. Infect. (2016).

C.H. Lai, J.C. Huang, H.H. Cheng, M.C. Wu, M.Z. Huang, H.H. Hu, et al., Helicobacter pylori cholesterol glucosylation increases intracellular survival in macrophages, Cell. Microbiol. (2018) e12947.

P. Morey, L. Pankuch, E. Pang, F. Roccella, M. Sigal, A. Imai-Matsushima, et al., Helicobacter pylori depletes cholesterol in gastric glands to prevent interferon gamma signaling and escape the inflammatory response, Gastroenterology 154 (5) (2018) 1391-404.e8.

M.A. Garia, N. Kumar, A. Hussain, S. Suman, S.N. Doddam, L.P. Sepe, et al., Roles of cholesteryl-sulfonate-Glucose transferase and cholesteryl glucosides in main tenance of Helicobacter pylori morphology, cell wall integrity, and resistance to antibiotics, MBio 9 (6) (2018).

F. Megraud, V. Neman-Simha, D. Brugmann, Further evidence of the toxic effect of ammonia produced by Helicobacter pylori urease on human epithelial cells, Infect. Immun. 60 (5) (1992) 1858–1863.

M. Takahashi, S. Miura, Y. Koura, T. Fukumura, I. Kuruse, H. Suzuki, et al., Helicobacter pylori-associated ammonia production enhances neutrophil-dependent gastric mucosal cell injury, Am. J. Pathol. 263 (5 Pt 1) (1992) G719–25.

H. Gu, Role of flagella in the pathogenesis of Helicobacter pylori, Curr. Microbiol. 74 (7) (2017) 863–869.

G. Geis, S. Suerbaum, B. Fornhoff, H. Levyng, W. Opferkuch, Ultrastructure and biochemical studies of the flagellar sheath of Helicobacter pylori, J. Med. Microbiol. 38 (5) (1993) 371–377.

D. Roncarati, V. Scarselli, Single molecule display by two transcriptional repressors and choraeones orchestrates Helicobacter pylori heat-shock response, Int. J. Mol. Sci. 19 (6) (2018).

E.L. Eskinstein, P. Saltif, Autophagy: a lysosomal degradation pathway with a central role in health and disease, Biochim. Biophys. Acta 1793 (4) (2009) 664–673.

D.J. Klionsky, The molecular machinery of autophagy: unanswered questions, J. Cell. Sci. 118 (Pt 1) (2005) 7–18.

A.C. Massey, C. Zhang, A.M. Cuervo, Chaperone-mediated autophagy in aging and disease, Curr. Top. Dev. Biol. 73 (2006) 205–235.

E. Bejarano, A.M. Cuervo, Chaperone-mediated autophagy, Proc. Am. Thorac. Soc. 7 (1) (2010) 29–39.

Z. Yang, D.J. Klionsky, An overview of the molecular mechanism of autophagy, Curr. Top. Microbiol. Immunol. 335 (2009) 3–12.

A.L. Anding, E.H. Barberee, Cleaning house: selective autophagy of organelles, Dev. Cell 15 (1)(2008) 344–357.

B. Levine, G. Kroemer, Autophagy in the pathogenesis of disease, Cell 132 (1) (2008) 27–42.

F. Geconi, B. Levine, The role of autophagy in mammalian development: cell death versus survival, Dev. Cell 15 (1) (2008) 344–357.

M. Wang, G. Hsijenhallis, Lipid raft-dependent uptake, signalling and in tracellular fate of Porphyromonas gingivalis in mouse macrophages, Cell. Microbiol. 13 (10) (2011) 2029–2043.

P.R. Sanders, P.R. Gibson, G.T. Cantin, D.C. Greenbaum, T. Nehr, D.J. Carucci, et al., Distinct protein classes including novel merovirose surface antigens in gat-like membrane of Plasmodium falciparum, J. Biol. Chem. 280 (48) (2005) 40169–40176.

K.A. Yoneayama, A.K. Tanaka, T.G. Silveria, H.K. Takahashi, A.H. Strauss, Characterization of Leishmania (Viamia) braziliensis membrane microdomains, and their role in macrophage infectivity, J. Lip. Res. 47 (10) (2006) 2171–2178.

M.C. Fernandes, M. Cortes, K.A. Geraldino Yoneayama, A.H. Strauss, N. Yoshida, R.A. Mortara, Novel strategy in Trypanosoma cruzi cell invasion: implication of cholesterol and host cell microdomains, Int. J. Parasit. 37 (13) (2007) 1431–1441.

M.L. Dykstra, R. Longernecker, S.K. Pierce, Epstein-Barr virus coopts lipid rafts to block the signaling and antigen transport functions of the BCR, Immunity 14 (1) (2001) 57–67.

D.R. Graham, E. Chertova, J.M. Hilburn, L.O. Arthur, J.E. Hildreth, Cholesterol depletion and immunomodulation: Evidence of genetic disease involving beta-cyclohexatin inactuates and permeabilizes the virus: evidence for a viro-associating lipid rafts, J. Virol. 77 (15) (2003) 8237–8248.

D.R. Taylor, I.J. Whitehouse, N.M. Hooper, Glypican-1 mediates both prion protein lipid raft association and disease isoform formation, PLoS Pathog. 5 (11) (2009) e1000666.

J.S. Wadis, M. Schäller, R.A. Williams, S.F. Dowdy, Pathological prion protein infects cells by lipid raft-dependent macropinocytosis, PLoS One 3 (10) (2008) e3314.

L.J. Pike, Lipid rafts: bringing order to chaos, J. Lip. Res. 44 (4) (2003) 655–667.

K. Simons, D. Toomre, Lipid rafts and signal transduction, Nat. Rev. Mol. Cell Biol. 1 (10) (2000) 31–45.

M.A. Alonso, J. Millan, The role of lipid rafts in signalling and membrane traffic, Curr. Microbiol. 45 (4) (2002) 229–235.

A. Lefkowitz, J.R. Feler, P. Holschneider, C. Ceng, H. Tapp, B. Busch, E. Loell, et al., Distinct protein classes including novel merozoite surface antigens in raft-mediated invasion of Plasmodium falciparum in T lymphocytes, J. Cell. Sci. 114 (Pt 22) (2001) 3957–3966.

T. Hanzal-Bayer, J.F. Hancock, Lipid rafts and membrane trafficking in T lymphocytes, J. Cell. Sci. 117 (15) (2004) 3175–3183.

H. Tshilima, H.K. Takahashi, A.H. Strauss, Immunomodulation and cell sorting in T lymphocytes, J. Cell. Sci. 117 (15) (2004) 3175–3183.

J.F. Beijer, M. Beijers, Cholesterol depletion reduces the TLR-4/LPS-mediated NF-kB activation in murine macrophages, J. Cell. Biochem. 103 (1) (2010) 233–245.

M.E. Athanasou, H. Kay, M. Salas, K. Depp, K. S, T. Li, S. McShane, et al., Distinct protein classes including novel merozoite surface antigens in raft-mediated invasion of Plasmodium falciparum in T lymphocytes, J. Cell. Sci. 114 (Pt 22) (2001) 3957–3966.

M. Kato, T. Kukita, T. Tsuchiya, H. Kato, A. Shirakawa, Y. Inawaya, et al., Distinct protein classes including novel merozoite surface antigens in raft-mediated invasion of Plasmodium falciparum in T lymphocytes, J. Cell. Sci. 114 (Pt 22) (2001) 3957–3966.
BIOL. 2 (2) (2001) 107–117.

[83] S.L. Schwartz, C. Cao, O. Pypppenko, A. Rak, A. Wandinger-Ness, RabGT Pases at a glance, J. Cell. Sci. 120 (Pt 22) (2007) 3905–3910.

[84] T. Galvez, J. Gillieron, M. Zerial, G.A. O’Sullivan, SnapShot: mammalian Rab proteins in endocytic trafficking, Cell 151 (1) (2012) 234–25.

[85] E.A. Roberts, J. Chua, G.B. Kyei, V. Deretic, Higher order Rab programming in phagosome-like biogenesis, J. Cell Biol. 174 (7) (2006) 923–929.

[86] G.B. Kyei, I. Verge, J. Chua, E. Roberts, J. Harris, J.R. Jumula et al., Rab14 is critical for maintenance of Mycobacterium tuberculosis phagosome-maturation arrest, EMBO J. 25 (22) (2006) 5250–5259.

[87] W. Zhao, T. Most, Y. Zhao, A.A. Guillhon, C. Buffat, J.P. Gorvel et al., The Salmonella effector protein SIdA plays a dual role in virulence, Sci. Rep. 5 (2015) 12979.

[88] E. Boucrot, T. Henry, J.P. Borg, J.P. Gorvel, S. Meresse, The intracellular fate of microbes in infected macrophage, Exp. Biol. Med. (2009).

[89] M.D. Welch, M. Way, Arp2/3-mediated actin-based motility: a tail of pathogen adaptation mechanism, Sci. Rep. (2016).

[90] M.G. Gutierrez, S.S. Master, S.B. Singh, G.A. Taylor, M.I. Colombo, V. Deretic, Role of lipids as phagosomal fate determinants, J. Clin. Invest. (2008) 2002–2011.

[91] I. Yamauchi, J. Elorza, V. Deretic, Secretory pathway in gastric epithelial cells, Autophagy (2009).

[92] D.H. Abdelaziz, et al., Autophagy stimulation by rapamycin suppresses lung inflammation and infection by MIR30B benet, J. Pathol. 244 (4) (2018) 432–442.

[93] H. Tsugawa, H. Mori, M. Matsuoka, S. Sato, Y. Saito, M. Imoto et al., CAPZA1 determines the risk of gastric carcinogenesis by inhibiting Helicobacter pylori CagA-degraded autophagy, Autophagy 15 (2) (2019) 242–258.

[94] H. Tsugawa, C. Kata, H. Mori, J. Matsuoka, K. Kamayama, H. Saya et al., Cancer stem-cell marker CD44v9-positive cells arise from Helicobacter pylori-infected CAPZA1-overexpressing cells, Cell. Mol. Gastroenterol. Hepatol. 8 (3) (2019) 319–334.

[95] K. Hirata, H. Suzuki, H. Inanera, J. Matsuura, H. Tsugawa, O. Nagano, et al., CD44 variant 9 expression in primary early gastric cancer as a predictive marker for recurrence, Br. J. Cancer 109 (2) (2013) 379–386.

[96] M. Fukata, M.T. Abreu, Role of Toll-like receptors in gastrointestinal malignancies, Oncogene (2008).

[97] C. Wunder, Cholesterol glycosylation glucosylation promotes H pylori ulcer infection, Nat. Med. (2006).

[98] P. Rosenstiel, S. Hellmig, J. Hampe, S. Ott, A. Till, W. Fischbach et al., Influence of polymorphisms in the NOD1/CARD4 and NOD2/CARD15 genes on the clinical outcome of Helicobacter pylori infection, Cell. Microbiol. (2006).

[99] M. Ogawa, T. Yoshimori, T. Suzuki, H. Sagar, N. Mizushima, C. Sakanawa, Escape of intracellular Shigella from autophagy, Science 307 (5710) (2005) 727–731.

[100] T. Starr, R. Child, T.D. Wehrly, B. Hansen, S. Hwang, C. Lopez-Otin et al., Selective subversion of autophagy complexes facilitates completion of the Brucella intracellular cycle, Cell Host Microbe 11 (2) (2013) 32–45.

[101] C.A. Collins, A. De Maziere, S. Van Dijk, F. Carlsson, J. Klumperman, E.J. Brown, Ang-2 independent sequestration of ubiquitinated mycobacteria, PLoS Pathog. 5 (5) (2009) e1000430.

[102] C.L. Birmingham, V. Canadien, E. Gouin, E.B. Troy, J.P. Cossart, T. Yoshimori et al., Virulence of intracellular Salmonella depends on the recruitment of kinesin, Science 306 (5698) (2004) 1037–1040.

[103] H. Tsugawa, H. Mori, M. Matsuoka, S. Sato, Y. Saito, M. Imoto et al., CAPZA1 determines the risk of gastric carcinogenesis by inhibiting Helicobacter pylori CagA-degraded autophagy, Autophagy 15 (2) (2019) 242–258.

[104] H. Tsugawa, C. Kata, H. Mori, J. Matsuoka, K. Kamayama, H. Saya et al., Cancer stem-cell marker CD44v9-positive cells arise from Helicobacter pylori-infected CAPZA1-overexpressing cells, Cell. Mol. Gastroenterol. Hepatol. 8 (3) (2019) 319–334.

[105] K. Hirata, H. Suzuki, H. Inamada, J. Matsuura, H. Tsugawa, O. Nagano, et al., CD44 variant 9 expression in primary early gastric cancer as a predictive marker for recurrence, Br. J. Cancer 109 (2) (2013) 379–386.

[106] M. Fukata, M.T. Abreu, Role of Toll-like receptors in gastrointestinal malignancies, Oncogene (2008).

[107] C. Wunder, Cholesterol glycosylation glucosylation promotes H pylori ulcer infection, Nat. Med. (2006).

[108] P. Rosenstiel, S. Hellmig, J. Hampe, S. Ott, A. Till, W. Fischbach et al., Influence of polymorphisms in the NOD1/CARD4 and NOD2/CARD15 genes on the clinical outcome of Helicobacter pylori infection, Cell. Microbiol. (2006).

[109] M. Ogawa, T. Yoshimori, T. Suzuki, H. Sagar, N. Mizushima, C. Sakanawa, Escape of intracellular Shigella from autophagy, Science 307 (5710) (2005) 727–731.

[110] T. Starr, R. Child, T.D. Wehrly, B. Hansen, S. Hwang, C. Lopez-Otin et al., Selective subversion of autophagy complexes facilitates completion of the Brucella intracellular cycle, Cell Host Microbe 11 (2) (2013) 32–45.

[111] C.A. Collins, A. De Maziere, S. Van Dijk, F. Carlsson, J. Klumperman, E.J. Brown, Ang-2 independent sequestration of ubiquitinated mycobacteria, PLoS Pathog. 5 (5) (2009) e1000430.

[112] C.L. Birmingham, V. Canadien, E. Gouin, E.B. Troy, T. Yoshimori, P. Cossart, T. Yoshimori et al., Virulence of intracellular Salmonella depends on the recruitment of kinesin, Science 306 (5698) (2004) 1037–1040.

[113] C.L. Birmingham, V. Canadien, E. Gouin, E.B. Troy, T. Yoshimori, P. Cossart, T. Yoshimori et al., Virulence of intracellular Salmonella depends on the recruitment of kinesin, Science 306 (5698) (2004) 1037–1040.

[114] C.L. Birmingham, V. Canadien, E. Gouin, E.B. Troy, T. Yoshimori, P. Cossart, T. Yoshimori et al., Virulence of intracellular Salmonella depends on the recruitment of kinesin, Science 306 (5698) (2004) 1037–1040.