Reduced lysosomal clearance of autophagosomes promotes survival and colonization of Helicobacter pylori

Reduced lysosomal clearance of autophagosomes promotes survival and colonization of *Helicobacter pylori*

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Abstract

Evasion of autophagy is key for intracellular survival of bacteria in host cells, but its involvement in persistent infection by *Helicobacter pylori*, a bacterium identified to invade gastric epithelial cells, remains obscure. The aim of this study was to functionally characterize the role of autophagy in *H. pylori* infection. Autophagy was assayed in *H. pylori*-infected human gastric epithelium and

the functional role of autophagy was determined via genetic or pharmacologic ablation of autophagy in mouse and cell line models of *H. pylori* infection. Here we showed that *H. pylori* inhibited lysosomal function and thereby promoted the accumulation of autophagosomes in gastric epithelial cells. Importantly, inhibiting autophagosome formation by pharmacological inhibitors or genetic ablation of BECN1 or ATG5 reduced *H. pylori* intracellular survival, whereas inhibition of lysosomal functions exerted an opposite effect. Further experiments demonstrated that *H. pylori* inhibited lysosomal acidification and the retrograde trafficking of mannose-6-phosphate receptors, both of which are known to positively regulate lysosomal function. We conclude that *H. pylori* subverts autophagy into a pro-survival mechanism through inhibition of lysosomal clearance of autophagosomes. Disruption of autophagosome formation offers a novel strategy to reduce *H. pylori* colonization in human stomachs.

Keywords: *Helicobacter*; xenophagy; subversion; lysosome

Introduction

Helicobacter pylori, one of the most prevalent infectious pathogens in the world, is a major trigger for pathological changes in the gastric mucosa, including gastritis, intestinal metaplasia, dysplasia and adenocarcinoma [1,2]. Successful colonization of *H. pylori* in the hostile environment of gastric mucosa relies on adaptation of the bacteria through multiple cellular and molecular mechanisms, including urease-mediated acid resistance [3], cell motility [4], and adherence to epithelial cells [5]. *H. pylori* also displays invasive properties and can survive inside gastric epithelial cells [6]. The effect of host factors on the colonization of *H. pylori*, however, remains poorly understood.

Autophagy is a stepwise process that begins with the sequestration of intracellular materials by the double-membrane autophagosomes, followed by autophagosome-lysosome fusion and lysosome-dependent degradation. Xenophagy is a specialized form of autophagy that degrades intracellular pathogens. In recent years, xenophagy has increasingly been recognized as a key mechanism for defending against bacterial, viral and parasite infections [7]. In this connection, xenophagy confers protection against numerous intracellular bacteria through direct destruction of internalized bacteria [8]. For instance, autophagy activation by different stimuli, such as nutrient starvation,

interferon-γ or vitamin D, could inhibit the survival of intracellular *Mycobacterium tuberculosis* [9]. Facultative intracellular pathogens, such as *Salmonella enterica* and *Serovar typhimurium*, are also eliminated through xenophagy via stimulating phosphorylation of the autophagy receptor optineurin [10].

Autophagy has been promulgated as an adaptive host response to *H. pylori* infection. Autophagy can be induced by *H. pylori* in cultured human gastric epithelial cells [11] and macrophages [12]. Autophagy also functions as a major pathway for degrading vacuolating cytotoxin A (VacA) [13] and cytotoxin-associated gene A (CagA) [14], both of which are critical virulence factors of *H. pylori*. Interestingly, recent studies revealed that *H. pylori* could modulate autophagy through deregulation of microRNAs [15,16]. People carrying a Crohn's-disease-associated single-nucleotide polymorphism in the autophagy gene *ATG16L1* also have increased susceptibility to *H. pylori* infection [13]. Herein, we demonstrate *in vitro* and *in vivo* that *H. pylori* can subvert autophagosomes into shelters by inhibiting lysosomal clearance.

Materials and Methods

Clinical specimens

Twelve *H. pylori*-positive and 7 *H. pylori*-negative gastric tissues were collected from patients who underwent gastric biopsy procedures at the Prince of Wales Hospital (Hong Kong SAR, P.R. China). The diagnoses were based on clinical and histological laboratory examination. All patients gave written informed consent for the use of clinical specimens for research purposes. This study was approved by the Joint CUHK–NTEC Clinical Research Ethics Committee. More detailed information is shown in supplementary material, Table S1.

Cell & bacterial culture

The human gastric epithelial cell line HFE-145 was a gift from Prof. Hassan Ashktorab and D. Smoot (Howard University, Washington, USA). Murine embryonic fibroblasts (MEFs) *Atg5*$^{-/-}$ and *Atg5*$^{+/+}$ cells were a gift from Dr. Noboru Mizushima (Tokyo Medical and Dental University, Tokyo, Japan). HFE-145 and MEF cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco™, Thermo Fisher, San Jose, CA, USA) supplemented with

10% fetal bovine serum (FBS) (Gibco™, Thermo Fisher) at 37°C in 5% CO_2. *H. pylori* strains SS1 (Sydney strain 1), TN2GF4 wild type and CagA knockout strain, and 4 clinically isolated strains (11639, 11644, 11666, 11667) were obtained from the Department of Microbiology, The Chinese University of Hong Kong. *H. pylori* was initially grown on horse blood agar plates (Columbia Blood Agar Base with DENT Selective Supplements (Oxoid, Basingstoke, United Kingdom) in an anaerobic jar with a microaerophilic environment for 5 d at 37 °C).

Experimental animals

C57BL/6 male mice of 6-7-weeks of age were used for *H. pylori* infection *in vivo* unless otherwise specified. Autophagy-deficient (*B6.129X1-Becn1tm1Blev/J*) mice with exons 1 and 2 of the autophagy-related gene *Beclin1* replaced with a neomycin selection cassette (purchased from the Jackson Laboratory (Bar Harbor, Maine, USA)) and their corresponding age-matched wild-type littermates were housed in the Laboratory Animal Unit of the Chinese University of Hong Kong. The cages, bedding, food and water were all autoclaved and the animals were given free access to tap water. The room was maintained at 22 ± 1 °C and humidity was 65–70% on 12

h light / 12 h dark cycle. All animal experiments were approved by the Laboratory Animals Ethics Committee of The Chinese University of Hong Kong.

Murine H. pylori infection model

H. pylori were grown in brain heart infusion (BHI) broth supplemented with 15% FBS for 20–30 h at 37 °C under microaerobic conditions and saturated humidity, with shaking at 200 rpm. C57BL/6 mice were orally gavaged with 10^8 colony-forming units (CFU) of *H. pylori* SS1 or sterile BHI broth (as a control) using gastric intubation once daily (pre-treatment model) or on alternate days for a total of three doses (post-treatment model) at 8 weeks of age.

Western blots

Tissues or cells were lysed in radioimmunoprecipitation assay (RIPA) buffer containing 0.5% cholic acid, 0.1% sodium dodecyl sulphate, 2 mM EDTA, 1% Triton X-100, 10% glycerol, 1.0 mM phenylmethylsulphonyl fluoride and 1 μg/mL aprotinin. After sonication for 30 s on ice and centrifuging for 20 min at 12,000 rpm at 4 °C, the supernatant was collected and protein

concentration was determined by protein assay kit (Bio-Rad, Hercules, CA, USA), using bovine serum albumin (BSA) as the standard. Fifty micrograms of each protein sample was resolved using SDS polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were blotted onto nitrocellulose membranes (Bio-Rad). Non-specific binding was blocked in 5% blocking buffer (5% non-fat dry milk, 150 mM Tris-HCl, pH 7.4, 50 mM NaCl, 0.05% Tween 20) for 1 h, and membranes were incubated overnight at 4 °C with the indicated primary antibody at a 1:1000 dilution. The membranes were then incubated for 1 h with horseradish peroxidase-coupled secondary antibody at room temperature. Chemiluminescent signals were then developed with LumiGLO reagent (Cell Signaling Technology, Beverly, MA, USA) and detected on X-ray film (Fuji Photo Film Co., Ltd., Tokyo, Japan).

Statistical analysis

All data were expressed as the mean ± standard error of mean (S.E.M.). Differences between two groups were compared using the Mann-Whitney U test or Student's t test. Multiple group comparisons were made using the Kruskal-Wallis test or one-way analysis of variance

(ANOVA) followed by Tukey's t-test. P values less than 0.05 were considered statistically significant.

Results

Autolysosomal degradation function was impaired due to *H. pylori* infection

We first verified that an increased autophagosome number was present in *H. pylori*-infected human stomach biopsies and mouse stomachs. As revealed in Figure 1A-B, *H. pylori* infection significantly increased the number of LC3-positive spot-like structures (is a hallmark of autophagy) in human and mouse gastric mucosa. Additionally, normal human gastric epithelial cells (HFE-145) were transfected with GFP-tagged LC3B expression plasmid, followed by *H. pylori* co-incubation. Consistent with the human and animal results, *H. pylori* caused an increased number of intracellular LC3B-positive puncta in HFE-145 cells (Figure 1C). To further determine the effect of *H. pylori* infection on autophagy, another autophagy marker, SQSTM1/p62 was assessed. We observed a concomitant increased LC3B-II and SQSTM1/p62 protein levels in *H. pylori*-infected human stomach biopsies (Figure 1D). In addition, *H. pylori*-negative gastritis samples showed accumulation of LC3B-II and SQSTM1/p62 proteins as compared with *H. pylori*-negative non-

gastritis stomachs (supplementary materials, Figure S1), suggesting that other pathological factors might also be involved in autophagy-impairment in human stomachs. Consistent with human biopsies' results, we observed concomitantly increased LC3B-II and SQSTM1/p62 protein levels in *H. pylori*-infected mouse stomachs (Figure 1E) as well as HFE-145 cells (Figure 1F), indicating that the dynamic autophagy processes might be impaired at the late stage (i.e. autophagosome-lysosome fusion and/or lysosome-dependent degradation). Nevertheless, lysosome-associated membrane protein 2 (LAMP2) was observed to be colocalised with LC3B in *H. pylori*-infected HFE-145 cells (Figure 1G), ruling out the possibility that the autophagosome-lysosome fusion was impaired due to bacterial infection. Additionally, autophagic flux assay showed that *H. pylori* did not further increase LC3B-II protein levels upon treatment with Bafilomycin A1 (Baf A1; a selective inhibitor of vacuolar H^+-ATPase to prevent lysosomal acidification) (Figure 1H). Thus, these findings support that *H. pylori* infection caused the autolysosomal degradation function to be disrupted.

H. pylori infection inhibited lysosome function

In order to evaluate the impact of *H. pylori* infection on lysosomal function, we measured three lysosome enzyme activities, namely acid phosphatase [17], N-acetyl-β-D-glucosaminidase (β-

NAG) [18], and cathepsin D [19] in *H. pylori*-infected gastric epithelial cells. Infection of HFE-145 cells with *H. pylori* markedly reduced the activities of all three enzymes as compared with uninfected cells (Figure 2A, B, C). The reduced activity of β-NAG was further confirmed in *H. pylori*-infected murine gastric mucosa (Figure 2D; acid phosphatase and cathepsin D activities were not measured because insufficient amounts of tissue were available). Pro-cathepsin D, a short-lived inactive precursor ofthe lysosomal aspartyl protease, is cleaved into the mature form upon acidification in lysosomes. Therefore, the maturation of cathepsin D is considered one of the hallmarks of intact lysosomal degradative function [19]. Consistently, we observed a prominent accumulation of pro-cathepsin D protein in *H. pylori*-positive gastric biopsies as compared with *H. pylori*-negative samples (Figure 2E). Murine (Figure 2F) and cell line (Figure 2G) models showed concordant results. To further confirm whether the autophagic vacuoles containing *H. pylori* were indeed lacking in proteolytic activity, cathepsin B activity was measured with Magic Red staining. Results showed that most of Magic Red signals were colocalised with LAMP1 in HFE-145 cells (supplementary material, Figure S2), suggesting cathepsin B activity was restricted to lysosomes. Importantly, when cells infected with FITC-labelled *H. pylori* were stained with Magic Red, the fluorescence signals of Magic Red from *H. pylori*-positive puncta were substantially lower than that of *H. pylori*-negative puncta (Figure 2H), indicating a down-regulated proteolytic activity in

autolysosomes containing *H. pylori*. Taken together, these evidences indicated that *H. pylori* infection impaired lysosome function in gastric epithelial cells, mouse model and human stomach biopsies.

H. pylori infection disrupted lysosome acidification

Given the central role of a luminal acidic environment for lysosome function [20], we next determined whether *H. pylori* infection could alter lysosomal acidification using the Lysosensor Yellow/Blue DND-160 staining assay. As shown in Figure 3A, HFE-145 cells infected with *H. pylori* showed significantly elevated lysosomal pH as compared with control cells. Baf A1 was used as a positive control. To further examine the effect of *H. pylori* infection on lysosomal acidification, an acridine orange staining assay was used. This dye emits green fluorescence in the cytosol but its protonated form fluoresces bright red in acidic vesicular organelles [21]. Consistently, we observed a decreased number of red cytoplasmic puncta in HFE-145 cells upon *H. pylori* infection (Figure 3B), indicating that lysosomal acidification was compromised. Baf A1, used as a positive control, produced a similar staining pattern. The impairment of lysosomal acidification due to *H. pylori* infection was further confirmed by overexpressing the GFP-mCherry-LC3 fusion protein in HFE-145 cells [22]. Fluorescence from GFP but not mCherry is

quenched in acidic LC3B-positive autolysosomes (red colour) whereas non-acidic autophagosomes appear as yellow dots (GFP overlapped with mCherry signals). Using this approach, we found that HFE-145 cells infected with *H. pylori* or treated with Baf A1 showed an increased retention of non-acidic autophagosomes but very few acidic autolysosomes. In contrast, serum-starved HFE-145 cells showed that most LC3B-positive autophagic puncta lost GFP signal but retained mCherry signal, indicating an unimpeded lysosomal acidification (Figure 3C). Therefore, these data indicated that lysosomal acidification was disrupted in *H. pylori*-infected HFE-145 cells.

H. pylori repressed retrograde trafficking of mannose-6-phosphate receptors

The majority of lysosomal hydrolytic enzymes are synthesized in the trans-Golgi network (TGN), then transported to endosomes via both cation-dependent (CD-) and -independent (CI-) mannose-6-phosphate receptors (MPRs). Increasing acidity along the endocytic pathway (i.e. maturation of endosomes from early to late stage) then promotes the dissociation of hydrolases from their MPRs, which recycle back to the TGN [23]. We postulated that impairment of lysosome acidification by *H. pylori* could negatively impact on the retrograde trafficking of MPRs, which might perpetuate lysosome dysfunction via inhibiting the endocytic pathway. In this study, the amounts of TGN-co-localized CD- and CI-MPRs were measured in *H. pylori*-infected human gastric biopsies

using confocal microscopy. Consistent with our postulation, both CD- and CI-MPRs were colocalised with TGN in *H. pylori*-negative human gastric biopsies. However, both of them showed diffuse distribution in *H. pylori*-infected human gastric samples (Figure 4A). The same phenomenon was observed in *H. pylori*-infected HFE-145 cells. More importantly, the disrupted retrograde trafficking of MPRs was rectified by the enforced expression of Rab9 (Figure 4B), which mainly mediates the transport of MPRs from late endosomes to the TGN [24].

H. pylori was sequestered in autophagosomes in gastric epithelium

To ascertain whether *H. pylori* invading the gastric epithelial cells were localised inside autophagosomes, *H. pylori*-infected human gastric biopsies and HFE-145 cells were assessed using co-immunofluorescence staining. Results showed a significant co-localisation of *H. pylori* and LC3B-positive autophagic puncta in infected human stomachs (Figure 5A) and HFE-145 cells (Figure 5B). Parallel H&E-stained corresponding sections of human gastric mucosal biopsies are shown in Figure 5A. Consistent with this, confocal microscopy of 1-year *H. pylori* infected mice stomach sections showed a large proportion of *H. pylori* deep in the mucosa were colocalised with LC3B-positive puncta (Figure 5C), validating that *H. pylori* can invade intracellularly within the gastric epithelium.

Genetic or pharmacological inhibition of autophagosome formation reduces intracellular survival of *H. pylori*

Based on the above findings, we hypothesized that *H. pylori* might survive in non-degradative autophagosomes after sequestration. To test this hypothesis, we disrupted autophagosome formation to determine if it could enhance the clearance of intracellular *H. pylori*. After silencing the expression of Beclin-1 (a protein encoded by *BECN1* essential for autophagosome formation) using small interfering RNA (siRNA) in HFE-145 cells, intracellular *H. pylori* levels as measured by quantitative PCR and colony forming unit (CFU) assays were significantly reduced as compared to cells transfected with the control siRNA (Figure 6A, B). To corroborate this finding, wild type and *Becn1*$^{+/-}$ mice were orally gavaged with 10^8 CFU *H. pylori*. Chronically infected *Becn1*$^{+/-}$ mice displayed a reduced colonization of *H. pylori* as shown by immunofluorescence staining (Figure 6C), colony-formation (Figure 6D) and DNA measurement (Figure 6E). Similar to the effect of *BECN1* silencing, murine embryonic fibroblasts derived from *Atg5*-knockout mice (Atg5 is another protein essential for autophagosome formation) showed a decreased burden of intracellular *H. pylori* after co-incubation with the bacteria (Figure 6F). Autophagosome formation was further inhibited in *H. pylori*-infected HFE-145 cells using pharmacological inhibitors: 3-

methyladeneine (3-MA) and verteporfin, both of which are inhibitors of autophagosome formation [25,26], significantly enhanced the clearance of intracellular *H. pylori* in HFE-145 cells as shown by DNA levels (Figure 6G). Concordant with the *in vitro* data, mice treated with inhibitors of autophagosome formation (i.e. verteporfin and wortmannin [27]) displayed a reduced colonization of *H. pylori* after oral gavage with the bacteria as assessed using immunofluorescence staining (Figure 6H) and DNA measurement (Figure 6I). In addition, Western blots results showed that *H. pylori*-induced increase in LC3B-II levels were lowered by verteporfin or wortmannin (supplementary material, Figure S3A), suggesting the reduction of autophagosome number. Furthermore, although no obvious histopathologic change among experimental groups was observed (supplementary material, Figure S3B), the number of neutrophils infiltrating into mouse gastric mucosa was significantly higher in *H. pylori*-infected mice as compared to BHI group, whereas it was decreased after treatment with verteporfin or wortmannin (supplementary material, Figure S3C), suggesting that acute gastritis caused by *H. pylori* inoculation was repressed by inhibitors of autophagosome formation.

Interfering with lysosomal function increases the number of intracellular *H. pylori*

We next determined whether impairment of lysosome function could exert the opposite effect on intracellular *H. pylori* survival. Infected HFE-145 cells were treated with or without chloroquine (CQ, a selective inhibitor for lysosomal hydrolases and autophagosomal fusion and degradation) and concanamycin A (Con A, a more powerful V-ATPase inhibitor than Baf A1) [28]. Results showed that these two agents dramatically elevated intracellular *H. pylori* DNA levels in HFE-145 cells (Figure 6J).

Discussion

H. pylori is a Gram-negative bacterium that has colonized over 50% of the world's human population, and it is a strong risk factor for severe gastric disorders, including gastritis and gastric malignancy [2]. However, for uncertain reasons, our immune system constantly fails to eliminate this microbe. It has been proposed that autophagy may play a significant role in this process. In the present study, we verified that autophagosome number is increased upon *H. pylori* infection, consistent with multiple previous reports. Notably, we found a disrupting role of *H. pylori* infection on lysosomal proteolytic activities and autolysosomal degradation function, similar to the previous work done by Raju et al., which indicated a suppressive function of *H. pylori* cytotoxin VacA in autolysosomal maturation [13]. We also verified that *H. pylori*-containing autolysosomes are indeed

lacking in proteolytic activity as shown by co-immunofluorescence staining for Magic Red and LAMP1 (supplementary material, Figure S2) as well as Magic Red and FITC-labelled *H. pylori* (Figure 2H). It is well accepted that the associated autophagic reaction is mediated by VacA [29,30]. However, in the present study, we selected the SS1 strain, in order to construct a bacteria-colonized mouse model. It has been demonstrated that the s2/m2 type VacA expressed by the SS1 strain is less toxic [14]. Consistently, we observed a weakened inhibitory effect of s2/m2 type VacA on autolysosomal degradation induced by the SS1 strain as compared to the other five s1/m1 type strains, including TN2GF4 and four clinically-isolated strains (11639, 11644, 11666, 11667), confirming that VacA is involved in the disruption of autolysosomal system (supplementary material, Figure S4A, B). However, we also observed a similar disrupting effect of CagA in the TN2GF4 strain. In this connection, deletion of the CagA gene abrogated the disrupting effect (supplementary material, Figure S4C), suggesting that the suppressive function on autolysosomal maturation is due to a synergetic impact induced by several virulence factors of *H. pylori*, although the precise functions of CagA and VacA together with other virulence factors in the induction of autophagosome formation and impairment of lysosomal degradation still remain to be elucidated.

H. pylori is generally recognized as an extracellular bacterium adhering to the gastric epithelium surface, whereas several *in vitro* studies suggested the occurrence of bacteria invading into gastric epithelial cells [31-33]. In this study, we demonstrated the facultative intracellular nature of *H. pylori* using human stomach biopsies as well as mouse and gastric epithelial cell line models of *H. pylori* infection, proving that a proportion of *H. pylori* can invade into the gastric epithelium and survive within the dysfunctional autophagosomes. Importantly, reduced intracellular *H. pylori* survival was observed when we introduced siRNA against autophagy-related genes, or genetically ablated them, to inhibit autophagosome formation specifically. Therefore, the present findings may hint at the possible inefficient elimination of *H. pylori* by immune cells and extracellularly-acting antibiotics, because sequestered bacteria may survive and replicate in dysfunctional autophagosomes to avoid eradication. Mechanistically, we discovered that reduced lysosome acidification and disrupted retrograde trafficking of MPRs underlie lysosomal dysfunction in *H. pylori* infection.

Lysosomes are known as the primary degradative organelles in eukaryotic cells, which are essential for the efficient removal of damaged cellular materials, such as engulfed pathogens, to maintain the metabolic homeostasis. Its digestive function is executed by a number of hydrolytic enzymes, which

are activated and matured in a highly acidic condition (pH between 4.5 and 5.5) [34]. Therefore, normal lysosomal acidification is extremely important for its degradation function. Consistent with a previous work showing that VacA can neutralize endo/lysosomal pH [35], our study uncovered an inhibitory action of *H. pylori* infection on lysosomal acidification, thereby causing accumulation of non-digestive autophagosomes to benefit bacterial survival. Accordingly, impairment of lysosomal acidic environment by CQ and ConA resulted in enhanced intracellular *H. pylori* survival in cell model. CQ pre-treatment also significantly increased *H. pylori* colonization in the mouse stomachs (supplementary material, Figure S5), corroborating the substantial role of lysosomal acidification in *H. pylori* survival. Our present findings are reminiscent of a recent report that insufficient acidification of autolysosomes facilitates *Group A Streptococcus* survival and growth in endothelial cells [36]. However, the effect of *H. pylori* on lysosomal acidification might vary with experimental conditions. For instance, an earlier study showed an unaltered acidic pH within lysosomes as assessed using Oregon green-dextran after a 2 h invasion of AGS epithelial cells by VacA-positive *H. pylori* [37].

The digestive function of lysosomes relies on over 50 different hydrolytic enzymes. The transport of these hydrolases from the TGN through endosomes to lysosomes is dependent on an intact

trafficking system of MPRs [38]. In our study, we demonstrated that *H. pylori* infection hindered the retrograde transport of both CD- and CI-MPRs in human gastric biopsies and cultured gastric epithelial cells. Importantly, our findings further revealed that disrupted MPRs trafficking was recovered with Rab9 overexpression. A previous study showed that a similar effect could be induced by another intracellular bacteria *Salmonella enterica*, leading to the misrouting of newly synthesized lysosomal enzymes and thereby inhibiting lysosomal function to promote their own survival [39], suggesting a wider relevance of this machinery being targeted by intracellular bacteria to evade elimination.

Numerous studies have suggested that autophagy induction contributes to persistent infection of some bacteria, such as *Salmonella* and *Listeria monocytogenes*, in which inhibition of autophagosome formation suppresses bacterial replication inside host cells [40,41]. Atg16L1-hypomorphic mice also displayed enhanced survival upon infection with α-toxin-deficient *Staphylococcus aureus* [42] and were resistant to intestinal disease induced by *Citrobacter rodentium* [43]. Furthermore, the accumulation of non-degradative autophagosomes has been observed with some viruses for achieving autophagy subversion [44]. Similarly, our study demonstrated that genetic or pharmacological ablation of autophagosome

formation inhibited the intracellular survival of *H. pylori in vitro* and its colonization *in vivo*. Therefore, it is worthwhile to ascertain whether such inhibition could be effective as an antibacterial strategy when autophagy has already been subverted into a pro-bacterial mechanism. Paradoxically, several studies revealing that *H. pylori*-induced autophagy functions as an antibacterial mechanism have been published [12,13,45]. For instance, survival of *H. pylori* was increased in the $Atg5^{-/-}$ MEF [13], treatment with 3-MA [12] or inhibition of Atg12 and Beclin-1 expression by MIR30B [45]. Similar to the conflicting reports regarding the actions of *H. pylori* on lysosomal acidification, these discrepancies may be due to different experimental settings (e.g. different time-points).

Effective clearance of autophagic substrates is key to the anti-inflammatory and tumour-suppressive properties of autophagy. In this connection, the aggregation of excess p62/SQSTM1 has been shown to cause DNA damage and stimulate a pro-tumorigenic inflammatory response [46,47]. Therefore, it would be of interest to explore if autophagy subversion is mechanistically linked to *H. pylori*-associated chronic gastritis and gastric cancer.

In conclusion, we have shown that a novel repressive effect of *H. pylori* on lysosomal function is required for circumventing autophagic degradation, and this may be critical to the development of *H. pylori* persistent infection. Based on our results, it is expected that restoring lysosomal acidification or disrupting autophagosome formation will offer innovative approaches to treat *H. pylori* persistent infection in the future.

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Author contributions statement

LZ, WH performed experiments; All authors analysed the results; CKYC, JCYW, SCN, SHW collected clinical specimens; WKKW designed the study; CHC, MTVC and WKKW managed the project.

References

1. Cooke CL, An HJ, Kim J, *et al*. Modification of gastric mucin oligosaccharide expression in rhesus macaques after infection with Helicobacter pylori. *Gastroenterology* 2009; **137:** 1061–1071, 1071 e1061–1068.

2. O'Connor A, O'Morain CA, Ford AC. Population screening and treatment of Helicobacter pylori infection. *Nat Rev Gastroenterol Hepatol* 2017; **14:** 230–240.

3. Scott DR, Marcus EA, Weeks DL, *et al*. Mechanisms of acid resistance due to the urease system of Helicobacter pylori. *Gastroenterology* 2002; **123:** 187–195.

4. Specht M, Schatzle S, Graumann PL, *et al*. Helicobacter pylori possesses four coiled-coil-rich proteins that form extended filamentous structures and control cell shape and motility. *J Bacteriol* 2011; **193:** 4523–4530.

5. Liu T, Shi Y, Liu XF, *et al*. Helicobacter pylori HP0876 is dispensable for heme-iron acquisition but attenuates bacterial adherence to gastric epithelial cells. *Curr Microbiol* 2012; **65:** 254–261.

6. Necchi V, Candusso ME, Tava F, *et al*. Intracellular, intercellular, and stromal invasion of gastric mucosa, preneoplastic lesions, and cancer by Helicobacter pylori. *Gastroenterology* 2007; **132:** 1009–1023.

7. Orvedahl A, Levine B. Eating the enemy within: autophagy in infectious diseases. *Cell Death Differ* 2009; **16:** 57–69.

8. Tattoli I, Sorbara MT, Vuckovic D, *et al*. Amino acid starvation induced by invasive bacterial pathogens triggers an innate host defense program. *Cell Host Microbe* 2012; **11:** 563–575.

9. Deretic V, Singh S, Master S, *et al*. Mycobacterium tuberculosis inhibition of phagolysosome biogenesis and autophagy as a host defence mechanism. *Cell Microbiol* 2006; **8:** 719–727.

10. Wild P, Farhan H, McEwan DG, *et al*. Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. **Science** 2011; **333:** 228–233.

11. Terebiznik MR, Raju D, Vazquez CL, *et al*. Effect of Helicobacter pylori's vacuolating cytotoxin on the autophagy pathway in gastric epithelial cells. *Autophagy* 2009; **5:** 370–379.

12. Wang YH, Wu JJ, Lei HY. The autophagic induction in Helicobacter pylori-infected macrophage. *Exp Biol Med (Maywood)* 2009; **234:** 171–180.

13. Raju D, Hussey S, Ang M, *et al*. Vacuolating cytotoxin and variants in Atg16L1 that disrupt autophagy promote Helicobacter pylori infection in humans. *Gastroenterology* 2012; **142:** 1160–1171.

14. Tsugawa H, Suzuki H, Saya H, *et al*. Reactive oxygen species-induced autophagic degradation of Helicobacter pylori CagA is specifically suppressed in cancer stem-like cells. *Cell Host Microbe* 2012; **12:** 764–777.

15. Yang XJ, Si RH, Liang YH, *et al*. Mir-30d increases intracellular survival of Helicobacter pylori through inhibition of autophagy pathway. *World J Gastroenterol* 2016; **22:** 3978–3991.

16. Wu K, Zhu C, Yao Y, *et al*. MicroRNA-155-enhanced autophagy in human gastric epithelial cell in response to Helicobacter pylori. *Saudi J Gastroenterol* 2016; **22:** 30–36.

17. Braun M, Waheed A, von Figura K. Lysosomal acid phosphatase is transported to lysosomes via the cell surface. *EMBO J* 1989; **8:** 3633–3640.

18. Appelqvist H, Waster P, Kagedal K, *et al*. The lysosome: from waste bag to potential therapeutic target. *J Mol Cell Biol* 2013; **5:** 214–226.

19. Gieselmann V, Hasilik A, von Figura K. Processing of human cathepsin D in lysosomes in vitro. *J Biol Chem* 1985; **260:** 3215–3220.

20. Mindell JA. Lysosomal acidification mechanisms. *Annu Rev Physiol* 2012; **74:** 69–86.

21. Wu YC, Wu WK, Li Y, *et al*. Inhibition of macroautophagy by bafilomycin A1 lowers proliferation and induces apoptosis in colon cancer cells. *Biochem Biophys Res Commun* 2009; **382:** 451–456.

22. Qiu J, Tsien C, Thapalaya S, *et al*. Hyperammonemia-mediated autophagy in skeletal muscle contributes to sarcopenia of cirrhosis. *Am J Physiol Endocrinol Metab* 2012; **303:** E983–993.

23. Ghosh P, Dahms NM, Kornfeld S. Mannose 6-phosphate receptors: new twists in the tale. *Nat Rev Mol Cell Biol* 2003; **4:** 202–212.

24. Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. *Nat Rev Mol Cell Biol* 2009; **10:** 623–635.

25. Donohue E, Tovey A, Vogl AW, *et al*. Inhibition of autophagosome formation by the benzoporphyrin derivative verteporfin. *J Biol Chem* 2011; **286:** 7290–7300.

26. Wu YT, Tan HL, Shui G, *et al*. Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase. *J Biol Chem* 2010; **285:** 10850–10861.

27. Blommaart EF, Krause U, Schellens JP, *et al*. The phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 inhibit autophagy in isolated rat hepatocytes. *Eur J Biochem* 1997; **243:** 240–246.

28. Durrington HJ, Upton PD, Hoer S, *et al*. Identification of a lysosomal pathway regulating degradation of the bone morphogenetic protein receptor type II. *J Biol Chem* 2010; **285:** 37641–37649.

29. Raju D, Jones NL. Methods to monitor autophagy in H. pylori vacuolating cytotoxin A (VacA)-treated cells. *Autophagy* 2010; **6:** 138–143.

30. Yahiro K, Satoh M, Nakano M, *et al*. Low-density lipoprotein receptor-related protein-1 (LRP1) mediates autophagy and apoptosis caused by Helicobacter pylori VacA. *J Biol Chem* 2012; **287:** 31104–31115.

31. Dubois A, Boren T. Helicobacter pylori is invasive and it may be a facultative intracellular organism. *Cell Microbiol* 2007; **9:** 1108–1116.

32. Liu H, Semino-Mora C, Dubois A. Mechanism of H. pylori intracellular entry**:** an in vitro study. *Front Cell Infect Microbiol* 2012; **2:** 13.

33. Oh JD, Karam SM, Gordon JI. Intracellular Helicobacter pylori in gastric epithelial progenitors. *Proc Natl Acad Sci U S A* 2005; **102:** 5186–5191.

34. Xu H, Ren D. Lysosomal physiology. *Annu Rev Physiol* 2015; **77:** 57–80.

35. Satin B, Norais N, Telford J, *et al*. Effect of helicobacter pylori vacuolating toxin on maturation and extracellular release of procathepsin D and on epidermal growth factor degradation. *J Biol Chem* 1997; **272:** 25022–25028.

36. Lu SL, Kuo CF, Chen HW, *et al*. Insufficient acidification of autophagosomes facilitates Group A streptococcus survival and growth in endothelial cells. *MBio* 2015; **6:** e01435–01415.

37. Terebiznik MR, Vazquez CL, Torbicki K, *et al*. Helicobacter pylori VacA toxin promotes bacterial intracellular survival in gastric epithelial cells. *Infect Immun* 2006; **74:** 6599–6614.

38. Rohrer J, Kornfeld R. Lysosomal hydrolase mannose 6-phosphate uncovering enzyme resides in the trans-Golgi network. *Mol Biol Cell* 2001; **12:** 1623–1631.

39. McGourty K, Thurston TL, Matthews SA, *et al*. Salmonella inhibits retrograde trafficking of mannose-6-phosphate receptors and lysosome function. *Science* 2012; **338:** 963–967.

40. Yu HB, Croxen MA, Marchiando AM, *et al*. Autophagy facilitates Salmonella replication in HeLa cells. *MBio* 2014; **5:** e00865–00814.

41. Birmingham CL, Canadien V, Kaniuk NA, *et al*. Listeriolysin O allows Listeria monocytogenes replication in macrophage vacuoles. *Nature* 2008; **451:** 350–354.

42. Maurer K, Reyes-Robles T, Alonzo F, 3rd, *et al*. Autophagy mediates tolerance to Staphylococcus aureus alpha-toxin. *Cell Host Microbe* 2015; **17:** 429–440.

43. Marchiando AM, Ramanan D, Ding Y, *et al*. A deficiency in the autophagy gene Atg16L1 enhances resistance to enteric bacterial infection. *Cell Host Microbe* 2013; **14:** 216–224.

44. Gannage M, Dormann D, Albrecht R, *et al*. Matrix protein 2 of influenza A virus blocks autophagosome fusion with lysosomes. *Cell Host Microbe* 2009; **6:** 367–380.

45. Tang B, Li N, Gu J, *et al*. Compromised autophagy by MIR30B benefits the intracellular survival of Helicobacter pylori. *Autophagy* 2012; **8:** 1045–1057.

46. Mathew R, Karp CM, Beaudoin B, *et al*. Autophagy suppresses tumorigenesis through elimination of p62. *Cell* 2009; **137:** 1062–1075.

47. Duran A, Linares JF, Galvez AS, *et al*. The signaling adaptor p62 is an important NF-kappaB mediator in tumorigenesis. *Cancer Cell* 2008; **13:** 343–354.

*48. Zhang L, Yu J, Wong CC, *et al*. Cathelicidin protects against *Helicobacter pylori* colonization and the associated gastritis in mice. *Gene Ther* 2013; **20:** 751–760.

*49. Zhang L, Wu WK, Gallo RL, *et al*. Critical role of antimicrobial peptide cathelicidin for controlling *Helicobacter pylori* survival and infection. *J Immunol* 2016; **196:** 1799–1809.

*50. Wolfe DM, Lee JH, Kumar A, *et al*. Autophagy failure in Alzheimer's disease and the role of defective lysosomal acidification. *Eur J Neurosci* 2013; **37:** 1949-1961.

*Cited only in supplementary material.

Figure legends

Figure 1. Autolysosomal degradation function was impaired due to *H. pylori* infection. (A) *H. pylori*-negative (n = 6) and -positive (n = 12) human stomach biopsies were stained to visualize autophagosomes (LC3, green) and nuclei (DAPI, blue). Fifty visual fields from each group were randomly selected to quantitate LC3 positive puncta. Bars = 10 μm. (B) *H. pylori*-negative (n = 8) and -positive (n = 8) mouse stomachs were stained to visualize autophagosomes (LC3, green) and nuclei (DAPI, blue). Fifty visual fields from each group were randomly selected to quantitate LC3 positive puncta. Bars = 7.5 μm. (C) HFE-145 cells transfected with GFP-tagged LC3 plasmid were infected or not with *H. pylori* SS1 (MOI 100) for 3 h. Following a chase, in gentamicin-containing medium for 2 h and subsequent washes to remove extracellular *H. pylori*, incubation of cells continued for an additional 72 h with fresh medium. Twenty cells from each group were randomly selected to quantitate GFP-LC3-positive puncta. Bars = 10 μm. (D) *H. pylori*-negative (n = 6) and -positive (n = 12) human gastric biopsies were examined for LC3B and SQSTM1/p62 proteins. (E) C57BL/6 mice (n = 8 per group) were infected or not with *H. pylori* SS1 for 6 months. Three mice from each group were randomly selected to analyse LC3B and SQSTM1/p62 proteins in gastric tissues. (F) HFE-145 cells were infected or not with *H. pylori* SS1 as described in Figure 1C. LC3B-II and SQSTM1/p62 protein levels were quantified by Western blotting in three independent

experiments. (G) HFE-145 cells transfected with GFP-tagged LC3B plasmid were infected or not with *H. pylori* SS1 as described in Figure 1C. Over twenty cells from each group were randomly selected to determine the colocalisation coefficient (percentage of punctate LAMP2 signals that were positive for LC3B signals). Bars = 10 μm. (H) HFE-145 cells infected or not with *H. pylori* SS1 were treated with Baf A1 (100 nM, 48 h). LC3B protein was quantified in three independent experiments. Mean ± S.E.M. **, $p < 0.01$; ***, $p < 0.001$.

Figure 2. *H. pylori* infection suppressed lysosome acidification. (A - B) HFE-145 cells were infected or not with *H. pylori* SS1 as described in Figure 1C. Lysosome fractions were purified to determine acid phosphatase (A) and β-NAG (B) activities (n = 3). (C) Cathepsin D activity was measured in whole-cell lysates from control and *H. pylori* SS1-infected HFE-145 cells (n = 3). (D) β-NAG activity was determined in lysosome fractions isolated from control and 3 month-*H. pylori* SS1-infected mouse stomachs (n = 6 per group). (E) *H. pylori*-negative (n = 6) and -positive (n = 12) human gastric biopsies were examined and quantified for cathepsin D protein. (F) C57BL/6 mice (n = 8 per group) were infected or not with *H. pylori* SS1 for 6 months. Three mouse stomach tissues from each group were randomly selected to analyse cathepsin D protein. (G) HFE-145 cells were infected or not with *H. pylori* SS1 as described in Figure 1C. Cathepsin D proteins were examined

and quantified in three individual experiments. (H) HFE-145 cells treated with or without FITC-labelled *H. pylori* SS1 were stained with Cathepsin B substrate Magic Red. Representative images are shown. Magic Red fluorescence intensities for the H. pylori-infected group were determined by random selection of FITC-positive and negative signals from over 30 cells of multiple fields. Bars = 20 μm. Mean ± S.E.M. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 3. *H. pylori* infection disrupted lysosome acidification. (A - B) HFE-145 cells were infected or not with *H. pylori* SS1 as described in Figure 1C. (A) Lysosomal pH values were measured ratiometrically 160 from three individual experiments using Lysosensor Yellow/Blue DND. Baf A1 (100 nM; 24 h) was used as the positive control. (B) HFE-145 cells were labelled with 1 μM acridine orange (AO). Baf A1-treated cells (100 nM; 24 h) were used as positive controls. Bars = 50 μm. (C) HFE-145 cells expressing GFP-mCherry-LC3 fusion protein were infected or not with *H. pylori* SS1 as described in Figure 1C. Cells treated with serum-free medium (24 h) and Baf A1 (100 nM; 24 h) were used as negative and positive controls, respectively. Bars = 10 μm. Over twenty cells from each group were randomly selected to statistically analyse the colocalisation coefficient as the percentage of punctate mCherry (red) signals that were positive for GFP (green) signals. Mean ± S.E.M. **, $p < 0.01$; ***, $p < 0.001$.

Figure 4. *H. pylori* repressed retrograde trafficking of mannose-6-phosphate receptors. (A) *H. pylori*-negative (n=6) and –positive (n=12) human gastric biopsies were stained for TGN46 (green) / CD-MPR (red) (upper) or TGN46 (green) / CI-MPR (red) (bottom). Thirty visual fields from each group were randomly selected to analyse the colocalisation coefficient as the percentage of punctate signals of CD-MPR or CI-MPR that were positive for TGN46. (B) HFE-145 cells transfected with empty (pcDNA) or Rab9-encoding plasmid were challenged or not with *H. pylori* SS1 as described in Figure 1C. Cells were immunostained for TGN46 (green)/CD-MPR (red) (left) or TGN46 (green)/CI-MPR (red) (right). Colocalisation coefficients of TGN46 and CD-MPR or CI-MPR were statistically analysed as the percentage of punctate signals of TGN46 that were positive for CD-MPR or CI-MPR, determined using ImageJ software. Twenty visual fields from each group were randomly selected from three individual experiments. Mean ± S.E.M. *, $p < 0.05$; **, $p < 0.01$. Bars = 10 μm.

Figure 5. *H. pylori* was sequestered in autophagosomes in gastric epithelium. (A) *H. pylori*-negative (n = 6) and –positive (n = 12) human gastric biopsies were stained for LC3B (green), *H. pylori* (red) and DNA (blue). 30 visual fields per group were randomly selected to analyse the colocalisation coefficient as the percentage of punctate signals for LC3B that were *H. pylori* immunopositive.

Corresponding H&E stained sections are shown. Bars = 10 μm. (B) HFE-145 cells transfected with GFP-LC3 plasmid were infected or not with *H. pylori* SS1 as described in Figure 1C. Cells were then immunostained for LC3B (green) and *H. pylori* (red). Over twenty cells per group were randomly selected to analyse the localization coefficient (percentage of punctate signals of LC3B that were positive for *H. pylori*). Bars = 10 μm. (C) C57BL/6 mice (n = 8 per group) were infected or not with *H. pylori* SS1 for 1 year. Mouse stomachs from each group were stained for LC3B (green), *H. pylori* (red) and nuclei (blue). Thirty visual fields per group were randomly selected to determine the colocalisation coefficient (percentage of punctate signals of LC3B that were *H. pylori* immunopositive). Bars = 25 μm. Mean ± S.E.M. *, $p < 0.05$; **, $p < 0.01$.

Figure 6. Genetic or pharmacological inhibition of autophagosome formation reduced intracellular *H. pylori* survival. (A - B) HFE-145 cells transfected with control or Beclin-1-specific siRNA (siBECN-1) were infected or not with *H. pylori* SS1 as described in Figure 1C. Intracellular *H. pylori* levels were assessed using quantitative-PCR (A, *H. pylori*-specific 16S ribosomal DNA with GAPDH as the internal control) or by CFU assay (B) (n = 3). (C - E) Beclin-$1^{+/+}$ and Beclin-$1^{+/-}$ transgenic mice (n = 6 per group) were infected with *H. pylori* SS1 for 3 months. (C) *H. pylori*-negative and -positive mouse stomachs were stained for *H. pylori* (red) and nuclei (blue). Fifty visual

fields per group were randomly selected to count bacteria number. (D) Mouse gastric tissues were harvested, weighed, and homogenized in sterile PBS. Following serial dilution, samples were plated on *H. pylori*-selective blood agar plates and incubated for 1 week for colony counting. (E) *H. pylori* levels in mouse gastric tissue DNA were assessed by quantitative-PCR. (F) $Atg5^{+/+}$ and $Atg5^{-/-}$ MEFs were infected with *H. pylori* SS1 (MOI 100) for 3 h or 24 h. (Left) *H. pylori* levels assessed by quantitative-PCR. (Right) ATG5 and LC3B proteins were analysed (n = 3). (G) HFE-145 cells pre-treated with 3-MA (2 mM) or verteporfin (10 μM) for 1 h were infected with *H. pylori* SS1 as described in Figure 1C. Intracellular *H. pylori* were measured by quantitative-PCR (n = 3). (H - I) Mice (n = 8 per group) were pre-treated with autophagosome inhibitor, verteporfin (18 mg/kg i.p) or wortmannin (2 mg/kg i.p), for 7 days, then infected with *H. pylori* for 2 days. (H) *H. pylori*-negative and -positive mouse stomachs were stained for *H. pylori* (red) and nuclei (blue). Fifty visual fields per group were randomly selected to count bacteria number. (I) *H. pylori* DNA levels were assessed using quantitative-PCR. (J) HFE-145 cells pre-treated with chloroquine (CQ, 10 μM) or ConA (50 nM) for 1 h were infected with *H. pylori* SS1 as described in Figure 1C. Intracellular *H. pylori* DNA levels were assessed using quantitative-PCR (n = 3). Mean ± S.E.M. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

SUPPLEMENTARY MATERIAL ONLINE

Supplementary materials and methods YES

Supplementary figure legends YES

Figure S1. Human stomach biopsies were examined for LC3B, SQSTM1/p62 and GAPDH proteins

Figure S2. HFE-145 cells transfected with LAMP1-GFP (green) plasmid were stained with Magic Red (red) to visualize the colocalisation pattern in cells

Figure S3. Effects of verteporfin or wortmannin on LC3B and SQSTM1/p62 proteins in and neutrophil numbers in the stomachs of mice infected with *H. pylori* SS1 strain for 2 days

Figure S4. Levels of cathepsin D, SQSTM1/p62 and LC3B proteins in HFE-45 cells infected with strains of *H. pylori*, and analysis of their VacA and CagA genotypes

Figure S5. The effect of chloroquine pre-treatment on the growth of *H. pylori* in mouse stomachs

Table S1. Patients' clinical information