Involvement of autophagy in antibacterial actions of vitamin D in *Helicobacter pylori* infection: abridged secondary publication

W Hu, L Zhang, WKK Wu, CH Cho

KEY MESSAGES

1. We identified a unique pathogenic mechanism on how *Helicobacter pylori* can survive by hiding inside the autophagosomes in cells.
2. We discovered a novel antibacterial signalling pathway of VD3 through the activation of PDIA3/STAT3 - MCOLN3 - Ca2+ axis, to reactivate the lysosomal acidification and degradation function of autolysosomes, which is the key signal pathway for the antibacterial action of VD3 both in cells and in animals and perhaps further in humans.

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[1] **W Hu,** [1,2] **L Zhang,** [1,2] **WKK Wu,** [3,4] **CH Cho**

[1] *Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Hong Kong*
[2] *Institute of Digestive Diseases, State Key Laboratory of Digestive Diseases, LKS Institute of Health Sciences, CUHK Shenzhen Research Institute, The Chinese University of Hong Kong, Hong Kong*
[3] *School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong*
[4] *Laboratory of Molecular Pharmacology, Department of Pharmacology, School of Pharmacy, Southwest Medical University, Luzhou, Sichuan, China*

* Principal applicant and corresponding author: chcho@cuhk.edu.hk

Helicobacter pylori is a Gram-negative spiral bacterium that has colonised over 50% of the world's population as a result of constant failure in *H pylori* clearance by the host immune system in the upper gastrointestinal tract.[1] Worse, the globally accepted triple therapy (comprising a proton pump inhibitor plus two antibiotics) is challenged by a steady increase in *H pylori* resistance to classical antibiotics, especially to clarithromycin.[2] It has been suggested that the inefficacy in pathogen eradication is due to the capacity of *H pylori* for hiding inside host cells,[3,4] thereby escaping from the innate response by immune cells. Although the precise mechanisms of immune evasion remain obscure, increasing evidence suggests that autophagy plays an important role in the pathogenesis of *H pylori*–associated gastric disorders.[5,6]

Autophagy, characterised by the formation of double-membrane vesicles designated as autophagosomes, is an evolutionarily conserved self-degradation process utilised by host cells to maintain cellular homeostasis and protect against invading pathogens.[7] Lysosomes are membrane-bound organelles that contain over 50 different degradative hydrolases. By fusing with lysosomes, autophagosomes mature into autolysosomes, in which they sequester pathogens followed by degradation by lysosomal proteases.[8] Initially, the autophagic pathway was regarded as the host defence strategy against invading pathogens, as several microbes such as *Listeria monocytogenes* were captured by autophagosomes, and then degraded to avoid persistent infection.[9] However, emerging evidence underlines an unexpected behaviour of bacteria to disrupt such a defensive autophagic process, allowing active intracellular replication.[10] *H pylori* has been proved to induce autophagy in several gastric cell lines.[11] A suppressive function of *H pylori*–vacuolating cytotoxin in the autophagosome maturation was revealed,[12] suggesting that the bacterium might utilise these intracellular compartments to hibernate and further replicate for a long-term survival in the gastric mucosa. However, the molecular mechanisms by which *H pylori* evades the immune system and the effects of antibiotics need to be elucidated, and novel antibiotics are urgently demanded to efficiently eradicate *H pylori* infection through this unique cellular mechanism. In addition, these agents, if any, could provide a better alternative from the current traditional antibiotics for the treatment of drug-resistant *H pylori*.

In this study, we verified an occurrence of intracellular *H pylori* in normal human gastric epithelial HFE145 cells, and more important, inside the cells of human stomachs. Moreover, we observed that the pathogens were sequestered and survived in non-digestive autophagosomes, which is due to an impaired lysosomal acidification caused by *H pylori* infection. We further showed the involvement of *H pylori* virulence factor CagA in the inhibition of Ca^{2+} channel MCOLN3 protein. Thus, our current results might explain the inefficiency in *H pylori* clearance after the treatment with some membrane-impermeable antibiotics. The survival of bacteria in

disarmed autophagosomes might be a significant reason for *H pylori* to persist and recur in host stomachs.

Vitamin D3 (VD3) as a steroid hormone is known as a supplement to improve overall human health. Emerging evidence points out a potential antimicrobial activity of VD3 in humans. Indeed, its antimicrobial action against *Mycobacterium tuberculosis* infection has been verified,[13] even though the precise mechanisms governing this antibacterial activity remain controversial. In the current study, we reported an unexpected antimicrobial effect of VD3 against *H pylori* colonisation *in vitro* and in mouse stomachs. In contrast to conventional antimicrobial actions induced by VD3 through the production of antimicrobial peptides such as cathelicidin and β-defensin 2,[14] we proved that such antibacterial effect is mediated through PDIA3 receptor but not the classical vitamin D receptor. We further observed that 1,25D3, the active metabolite of VD3, initiated the nuclear translocation of PDIA3/STAT3 complex, and the subsequent transcriptional up-regulation of MCOLN3 channels. All these play a pivotal role in the antimicrobial action of VD3 against *H pylori* infection. MCOLN3 channel is predominantly expressed on the late endosomal and lysosomal membranes (>75%).[15] Ca^{2+} release from endolysosome via the MCOLN3 channel is necessary for the lysosomal acidification and maturation.[15,16] Our results showed a modulatory role of PDIA3/STAT3 complex in the MCOLN3 expression. Moreover, VD3 could up-regulate MCOLN3 channel, which is required for Ca^{2+} release from lysosomes, and then consequently normalised lysosomal acidification. Finally, the repaired digestive machinery operating inside the gastric cells would drive the bacteria to degradation through the revival autolysosomal pathway.

In conclusion, our study identified a unique pathogenic mechanism on how *H pylori* can survive by hiding inside the autophagosomes in cells. We discovered a novel antibacterial signalling pathway of VD3 through the activation of PDIA3/STAT3 - MCOLN3 - Ca2+ axis, to reactivate the lysosomal acidification and degradation function of autolysosomes (Fig), which is the key signal pathway for the antibacterial action of VD3 both in cells and in animals and perhaps further in humans. A

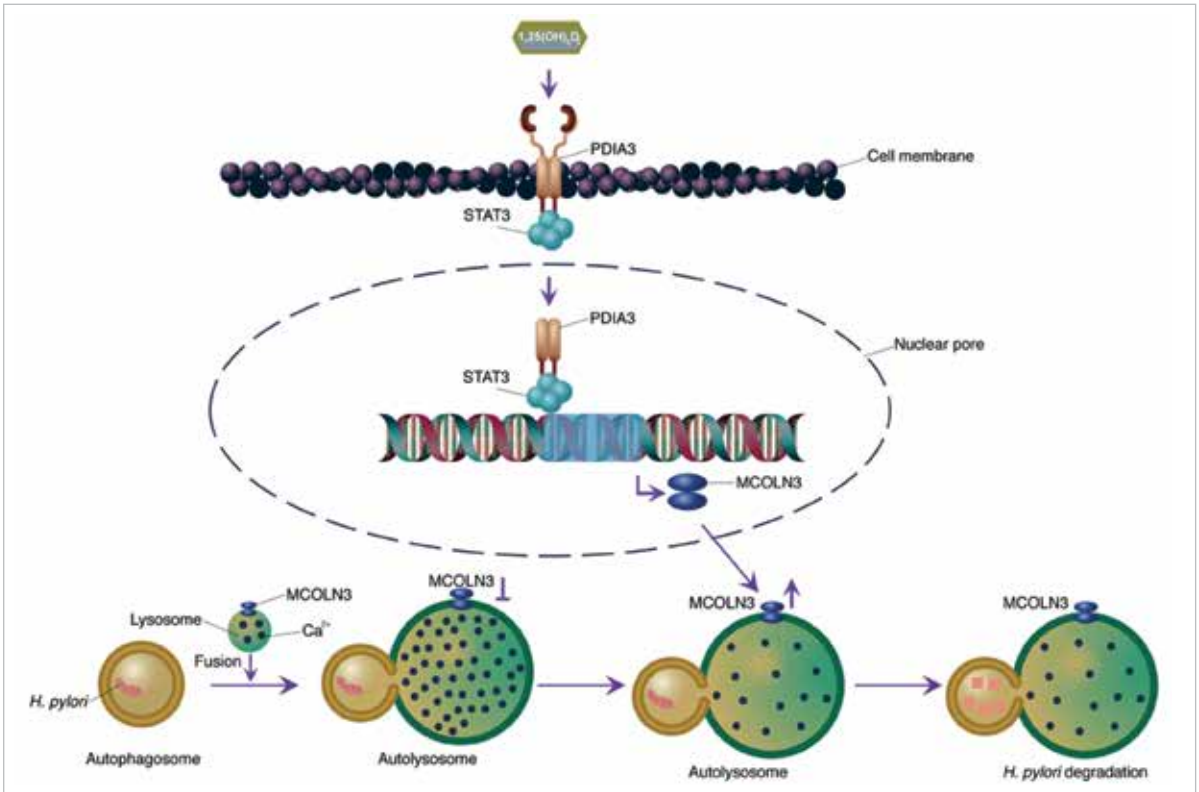

FIG. Schematic diagram depicting the proposed mechanism by which vitamin D3 exerts anti–*Helicobacter pylori* effects.
Autophagosomes enclosing *H pylori* were fused with lysosomes to form autolysosomes, whereas *H pylori* infection led to a down-regulated MCOLN3 protein level, resulting in an abnormal Ca^{2+} accumulation in lysosomes, and the impaired lysosomal acidification. Vitamin D3 treatment activated the membrane receptor PDIA3, drove the PDIA3/STAT3 complex redistributed into nucleus, causing an up-regulated MCOLN3 protein expression, thereby to recover the Ca^{2+} release from lysosomes followed by lysosomal acidification. As a consequence, *H pylori* was eliminated by the restored autolysosomal antibacterial activity.

clinical trial in the Prince of Wales Hospital, Hong Kong is currently underway to demonstrate a new therapeutic application of VD3 in *H pylori* infection and gastritis. Understanding of these mechanisms of action may provide new therapeutic strategies and targets and thereby new therapeutic agents for *H pylori* infection and treatment of its associated diseases in the upper gastrointestinal tract.

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Disclosure

The results of this research have been previously published in:
(1) Hu W, Zhang L, Li MX, et al. Vitamin D3 activates the autolysosomal degradation function against Helicobacter pylori through the PDIA3 receptor in gastric epithelial cells. Autophagy 2019;15:707-25.

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