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藥學碩士學位論文

Helicobacter pylori Infection Promotes Autophagy via Nrf2 Signaling in Human Gastric Cancer Cells

위장상피세포에서 헬리코박터 파이로리에 의한 오토파지 유도에 있어서 **Nrf2**의 역할

2015年 2月

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ABSTRACT

Helicobacter pylori Infection Promotes Autophagy via Nrf2 Signaling in Human Gastric Cancer Cells

JI YEON PAIK

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It has been reported that *Helicobacter pylori* (*H. pylori*) infection is one of the primary causes of gastritis and peptic ulcer diseases, which are provoked by oxidative stress and inflammation. More than 50% of the world's population is supposed to be infected by this bacterium. However, 90% of infected patients are asymptomatic, indicative of the existence of host defense mechanisms. Among them, nuclear factor-erythroid 2p45 (NF-E2)-related factor (Nrf2) is speculated to be involved in cellular defence against the *H. pylori*-induced gastritis. Autophagy, an autodigestive process that degrades cellular organelles and proteins, plays an important role in maintaining cellular homeostasis. To investigate the molecular mechanisms responsible for cellular response to *H. pylori*-induced gastric

inflammation, human gastric cancer cells (AGS cells) were infected with *H. pylori*. In this study, I found that *H. pylori* infection induces up-regulation of microtubule-associated light chain3 (LC3), an autophagic marker, by inducing accumulation of reactive oxygen species (ROS) and subsequently nuclear translocalization of Nrf2 in AGS cells. Notably, p62/SQSTM1, one of well-known autophagic substrates, regulated Nrf2 activation by *H. pylori*. Furthermore, Nrf2-induced LC3 up-regulation was mediated by heme oxygenase-1 and the generation of its by-product, carbon monoxide. *H. pylori* infection induced Nrf2 activation and p62 accumulation in C57BL6 female mice as well. Taken together, Nrf2 is considered to play a role in cellular adaptive response to *H. pylori*-induced gastritis by inducing autophagy.

Key Words

Helicobacter pylori, Nrf2, HO-1, ROS, LC3, Autophagy, Anti-apoptosis, Cancer cell survive

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Introduction

Multiple lines of evidence support that *Helicobacter pylori* infection is one of the primary causes of gastritis and peptic ulcer disease, which are provoked by oxidative stress. The majority of infected persons display a chronic superficial gastritis without clinical symptoms, although their gastric epithelium shows signs of inflammation (Morris, Ali et al., 1991, Blaser and Parsonnet 1994). According to the result of a 10 year's follow-up study with *Helicobacter pylori*-infected people, only 2.9% of *H. pylori* positive patients developed the gastric cancer, but the remaining individuals were asymptomatic. This observation suggests that there is the presence of host defense mechanisms against *H. pylori*-induced cellular damage, and among them, the transcription factor nuclear factor-erythroid 2p45 (NF-E2)-related factor (Nrf2) is speculated to play a key role.

Autophagy is an autodigestive process that degrades cellular organelles and proteins. It plays an important role in maintaining cellular homeostasis against environmental stress. Recent studies have shown an association of mammalian autophagy with neurodegenerative disease (Ralph et al., 2013), infectious disease (A Orvedahl et al., 2009), cardiovascular disease (Martinet W et al., 2007) and cancer (Robin Mathew et al., 2007). Microtubule-associated protein light chain 3 (LC3) is a soluble protein. During autophagy, a cytosolic form of LC3-l is conjugated to phosphatidylethanolamine to form LC3-phosphatidylethanolamine conjugate (LC3-II), which is recruited to autophagosomal membranes (Robin Mathew et al., 2007). It has been reported that *H. pylori* can manipulate the

autophagy of its host through production of the VacA virulence factor, and altered autophagy can influence intracellular survival and persistence of *H. pylori*. However, the detailed mechanism underlying *H. pylori*-induced autophagy is still unclear. In the present study, I attempted to investigate the role of Nrf2 in autophagic signal transduction which is activated by *H. pylori* infection.

The Keap1-Nrf2 pathway is the major regulator of cytoprotective responses to endogenous and exogenous stresses caused by reactive oxygen species (ROS) and electrophiles (Emilia Kansanen et al., 2012). Several studies have shown that high levels of reactive oxygen species (ROS) are harmful to normal cells and can cause tumor development by inducing DNA damage, increasing cancer-causing mutations, and activating inflammatory pathways. It was also known that ROS can trigger activation of Nrf2, a master regulator of the antioxidant response, (Gina M et al., 2011). In addition, ROS have an essential role in the regulation of autophagy (Scherz-Shouval R et al., 2011).

It has recently been reported that Nrf2 is activated through a non-canonical mechanism, which is p62/SQSTM1-dependent. p62/SQSTM1 is an ubiquitin-binding scaffold protein that co-localizes with ubiquitinated protein aggregates. The protein itself is degraded by autophagy and may link ubiquitinated proteins to autophagic machinery to enable their degradation in the lysosome (Roshan Ashoor, et al., 2013).

Among the target proteins induced by Nrf2, heme oxygenase-1 (HO-1) plays a pivotal role in cellular stress response. It was reported that HO-1 may contribute to

anti-oxidative and anti-inflammatory cytoprotection through the generation of its end products, such as carbon monoxide (CO) (Seon-jin Lee, et al., 2011). In this study, I investigated the role of CO overproduction by HO-1 activity as a consequence of Nrf2 activation plays a role in autophage induction by *H. pylori* infection.

Materials and Methods

Materials

Rabbit anti-LC3 (#2775), Rabbit anti-p62/SQSTM1 (#5114) Cell signaling Technology; rabbit anti-LC3 (NB100-2220), Novus Biologicals; rabbit anti-Nrf2 (sc-722), goat anti-Lamin B (sc-6216), rabbit anti-Actin (sc-1615), Santa Cruz Biotechnology Inc.; rabbit anti-HO-1 (ADI-SPA-895), Enzo Life Sciences, Inc.; Secondary antibodies for Western blotting were purchased from Zymed Lavoratories (SanFrancisco, CA, USA). Polyvinylidenedifluoride (PVDF) membrane was obtained from Gelman Laboratory (AnnArbor, MI, USA) and chemiluminescence (ECL) detection kit was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). LAS-4000 image reader was obtained from Fugi film (Tokyo, Japan).

Cell culture

AGS cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained in RPMI1640 (Gibco BRL) containing heat inactivated 10 % fetal bovine serum (FBS) and 1% of antibiotic-antimycotic mixture at 37 ℃ with 5% CO_2 and 95% air.

***Helicobacter pylori* culture**

H. pylori (ATCC 43504) with the typical S shape, gram negative rods, possessing the CagA, VacA, oxidase, urease, and catalase were provided in a frozen state by ATCC. Mouse adaptive strain of *H. pylori*, Sydney strain 1 (SS1) (Lee, O'Rourke et al., 1997) was provided (Gacheon Lee Gil Ya Cancer and Diabetes Institute). Both strains were cultured on tryptic soy agar with 5% sheep blood (BD Diagnostics) and Dent antibiotics supplement (Oxoid) at 37 °C under microaerophilic conditions (Campy-Pak System; BBL). Colonies were collected and suspended with Tryptic Soy Broth (BD Diagnostics) supplemented with 10% FBS. The number of bacterial cells in the suspension was counted by optical density measurement. The AGS cells were treated with 100 MOI (Multiple of Infection) of *H. pylori* ATCC43504 strain as $1x10^8$ CFUs (colony-forming units) to $1x10^6$ numbers of mammalian cells.

Transient transfection of small interfering RNA (siRNA) and expression vectors

The target sequences for Nrf2 siRNA were as follows: forward 5'AAG AGU AUG AGC UGG AAA AAC TT-3' and reverse 5'-GUU UUU CCA GCU CAU ACU CUU TT-3'. The target sequences for p62/SQSTM1 siRNA were as follows: 5'-GCA TTG AAG TTG ATA TCG AT-3'. Transfection was performed with Lipofectamine RNAiMAX (Invitrogen) for siRNA.

Reverse transcription-polymerase chain reaction analysis (RT-PCR)

Total RNA was isolated from AGS cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. To generate the cDNA from RNA, 1 g of total RNA was reverse transcribed with murine leukemia virus reverse transcriptase for 50 min at 42 °C and again for 15 min at 72 °C. One µl of cDNA was amplified with a PCR mixture in sequential reactions. The primers used for each RT-PCR reactions are as follows: human *HO-1*, 5'-CAG GCA GAG AAT GCT GAG TTC-3' and 5'-GAT GTT GAG CAG GAA CGC T-3'; human *Nrf2*, 5'-TTC AAA GCG TCC GAA CTC CA-3' and 5'-AAT GTC TGC GCC AAA AGC TG-3'; human *GAPDH*, 5'-AAG GTC GGA GTC AAC GGA TT-3' and 5'-GCA GTG GGT CTC TCT CCT-3'. Amplification products were analyzed by 2% agarose gel electrophoresis, followed by staining with SYBR Green and photographed using fluorescence in LAS-4000.

Immunocytochemical analysis

AGS cells were infected with *H. pylori* for the indicated intervals. After fixation with cold 95% MeOH/5% acetic acid for 10 min at 4 °C, samples were permeabilized with 0.2% Triton X-100 5 min at room temperature and then blocked with 5% bovine serum albumin in PBST (PBS containing 0.1% Tween-20) for 1 h at room temperature. Samples were then incubated with primary antibody

specific for LC3 overnight at 4 °C, followed by incubation with fluorescein isothiocyanate-goat anti-rabbit IgG secondary antibody for 1 h at room temperature. Images were assessed under a fluorescent microscopy (Nicon, Japan).

Statistical Analysis

Values were expressed as the mean +/- SE of at least three independent experiments. Statistical significance was determined by Student's *t* test and $P < 0.05$ was considered to be statistically significant.

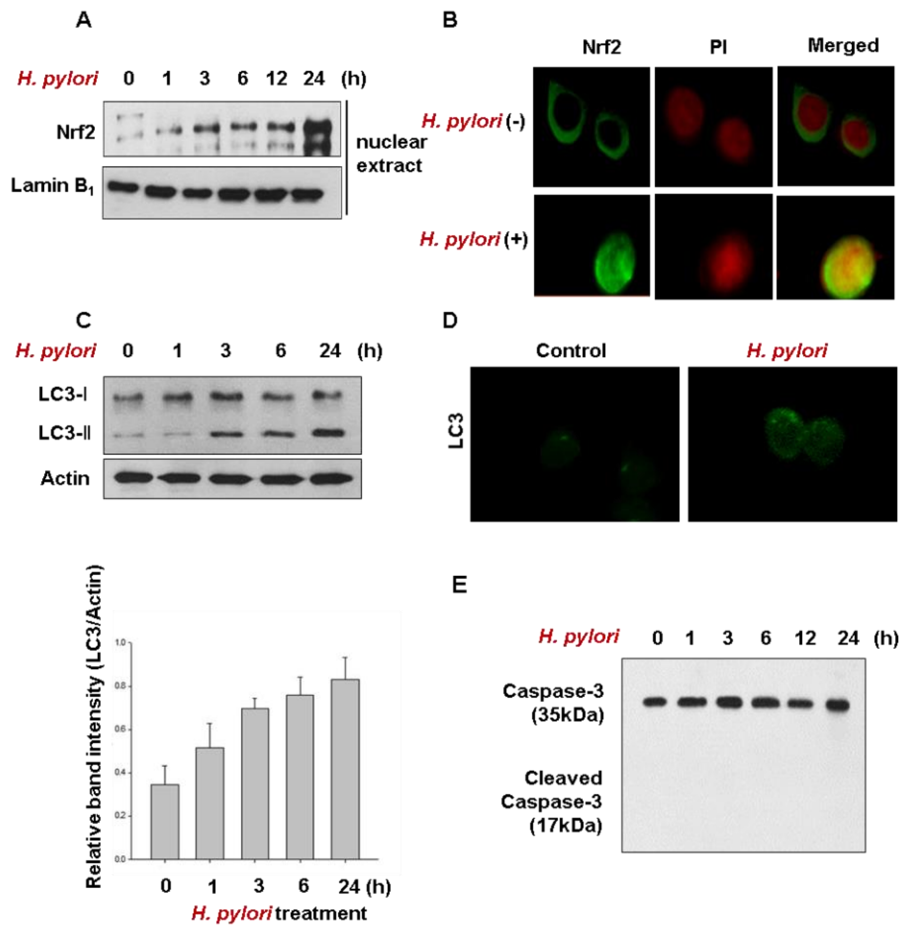
Results

H. pylori infection induces Nrf2 and LC3 expression in cultured AGS cells and in C57BL6 female mice *in vivo.*

Human gastric cancer cells (AGS cells) were co-incubated with *H. pylori* ATCC43504 strain. The nuclear localization of Nrf2 was transiently upregulated after 3 h of incubation (Fig. 1A). Immunocytochemical analysis also showed that the translocation of Nrf2 into the nucleus upon *H. pylori* infection (Fig. 1B). Autophagy is an autodigestive process that degrades cellular organelles and proteins. LC3 is a well-known autophagic marker**.** LC3 expression was upregulated in a time-dependent manner (Fig. 1C). In addition, LC3 dots surrounding autophagosomes formation was increased in the *H. pylori*-treated cells compared with the control (Fig. 1D). Although caspase-independent cell death is not well studied in comparison with classical caspase-dependent apoptosis, several mechanisms have been suggested (Broker LE et al., 2005). To find out if it is a caspase-dependent autophagic pathway or not, I examined the caspase-3 expression level following *H. pylori* infection. As the cleaved caspase-3 form was not detected (Fig. 1E), *H. pylori*–induced autophage is a caspase-independent.

Expression of Nrf2 and LC3 was also examined in a mouse model. C57BL/6 female mice were inoculated with SS1-mouse adaptive *H. pylori* strain 5 times at 48 h intervals, and after 75 weeks of the final inoculation, mouse tissues were collected to check the protein expression. Expression of both Nrf2 and LC3 was

significantly upregulated in *H. pylori* infected mice (Fig. 1F and G).


A
H. pylori 0 1 3 6 12 24 (h)
Nrf2
Lamin B1
nuclear extract
B
Nrf2
PI
Merged
H. pylori (-)
H. pylori (+)
C
H. pylori 0 1 3 6 24 (h)
LC3-I
LC3-II
Actin
D
Control
H. pylori
LC3
Relative band intensity (LC3/Actin)
0 1 3 6 24 (h)
H. pylori treatment
E
H. pylori 0 1 3 6 12 24 (h)
Caspase-3 (35kDa)
Cleaved Caspase-3 (17kDa)

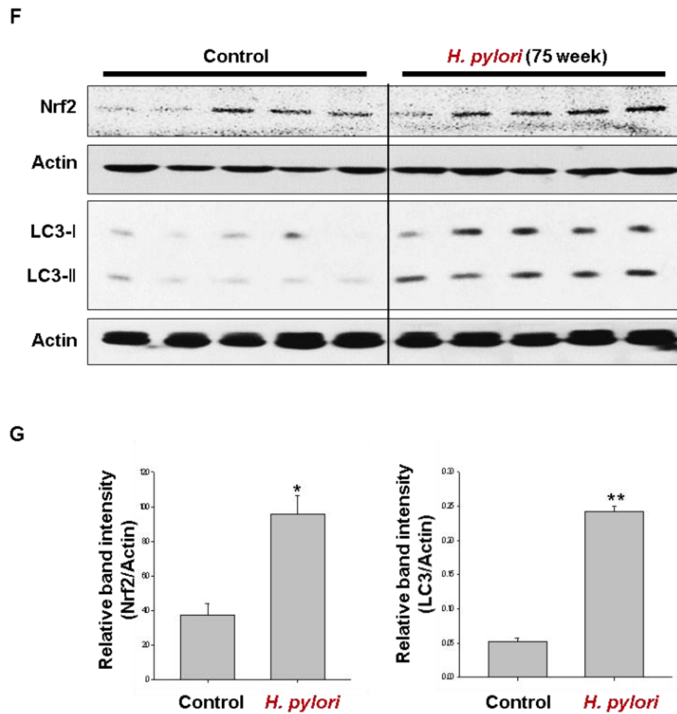


Figure 1. ***Helicobacter pylori*** **induces expression of Nrf2 and LC3 in cultured AGS cells and in C57BL6 female mice** ***in vivo***

AGS cells were inoculated with *H. pylori* ATCC43504 strain as 100 MOI for each indicated time. **A**. Nrf2 expression level **B**. Nrf2 translocation into the nucleus. **C**. Western blot analysis of LC3 protein expression in AGS cells. The relative expression levels of LC3 from three separate experiments are presented as means± S.D. **D**. Immunocytochemical analysis of LC3. (Arrow points LC3 dots) **E**. Caspase-independent autophagic process. C57BL/6 female mice were inoculated with 1×10^8 CFUs of *H. pylori* SS1, mouse adaptive strain five times 3 months

intervals. After 75 weeks of final inoculation, mice were sacrificed, and tissue lysates from mouse stomach were resolved by SDS-PAGE **F**. Western blot using antibodies against Nrf2 and LC3. **G**. The relative expression levels of Nrf2 and LC3 are presented as means±S.D. (n=5). *$p<0.05$

***H. pylori*-induced LC3 expression is regulated by Nrf2**

In order to determine whether LC3 is regulated by Nrf2, siRNA silencing of Nrf2 and Nrf2 knock out mice were utilized. The LC3 expression level in the Nrf2 silencing group was much lower upon *H. pylori* treatment than the control group (Fig. 2A). To support this result, immunocytochemical analysis was also performed using a LC3 antibody after transfecting AGS cells with Nrf2 siRNA followed by *H. pylori* infection for 24 h. As illustrated in Fig. 2B, there was decreased autophagosomes formation when Nrf2 was knocked down. Similarly, LC3 expression was abrogated in Nrf2 knock out mice (Fig. 2C) and this was verified by immunohistochemical staining (Fig. 2D). These data suggest that *H. pylori*-induced LC3 expression and autophage are likely to be mediated by Nrf2. In addition, Nrf2 knock out mice displayed severe inflammation compare to Nrf2 wild type mice following *H. pylori* infection (data not shown).

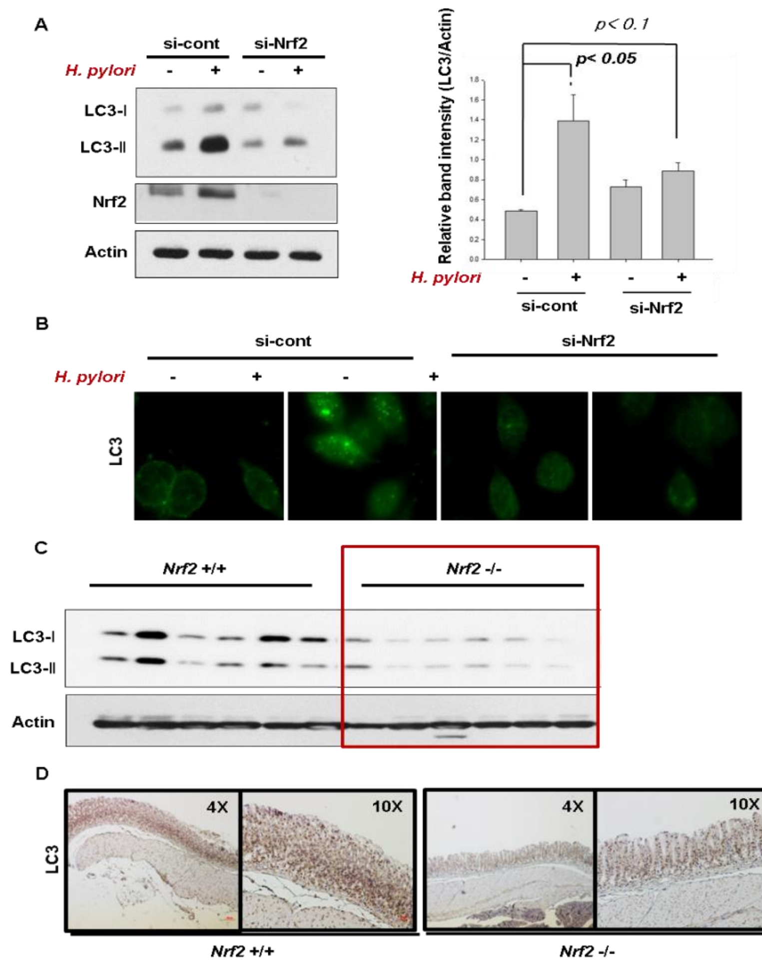


Figure 2. ***H. pylori*-induced LC3 expression is regulated by Nrf2.**

A. AGS cells were transiently transfected with specific small interfering RNA (siRNA) of Nrf2 using LipofectamineRNAiMAX followed by *H. pylori* treatment for 24 h. LC3 expression was abolished by Nrf2 siRNA. The expression level of LC3 is presented as means±S.D. $*P < 0.05$ **B**. Immunocytochemical analysis of LC3 **C**. The LC3 expression level of Nrf2 knockout mice was significantly reduced. **D**. Immunohistochemical analysis of LC3 in Nrf2 wild type and knockout mice.

***H. pylori*-enhanced ROS production was responsible for Nrf2 activation and LC3 induction**

Previous studies have demonstrated that the majority of stimuli that induce Nrf2 expression are caused by oxidative stress. Intracellular ROS accumulation resulting from exposure to *H. pylori* infection was measured using DCF-DA, a membrane permeable fluorescent probe that produces fluorescence upon reaction with peroxides such as H_2O_2. When AGS cells were incubated with *H. pylori* for 24 h, there was a marked increase in intracellular ROS accumulation and this was attenuated by pretreatment with the ROS scavenger NAC (Fig. 3A). Likewise, the upregulation of LC3 expression induced by *H. pylori* infection was abolished by NAC (Fig. 3B). These results suggest that *H. pylori* infection results in ROS production, which may, in turn, upregulate the conversion of LC3-I to LC3-II to promote autophagy via activation of Nrf2. Several papers reported that Nrf2 is activated through a non-canonical mechanism, which is p62/SQSTM1-dependent,.

p62/SQSTM1 is an ubiquitin-binding scaffold protein that co-localizes with ubiquitinated protein aggregates. In AGS cells infected by *H. pylori*, p62/SQSTM1 upregulated time dependently about in early time (Fig. 3C). When cells were transiently transfected with specific p62/SQSTM1 siRNA for 24 h, Nrf2 protein expression was abolished (Fig. 3D).

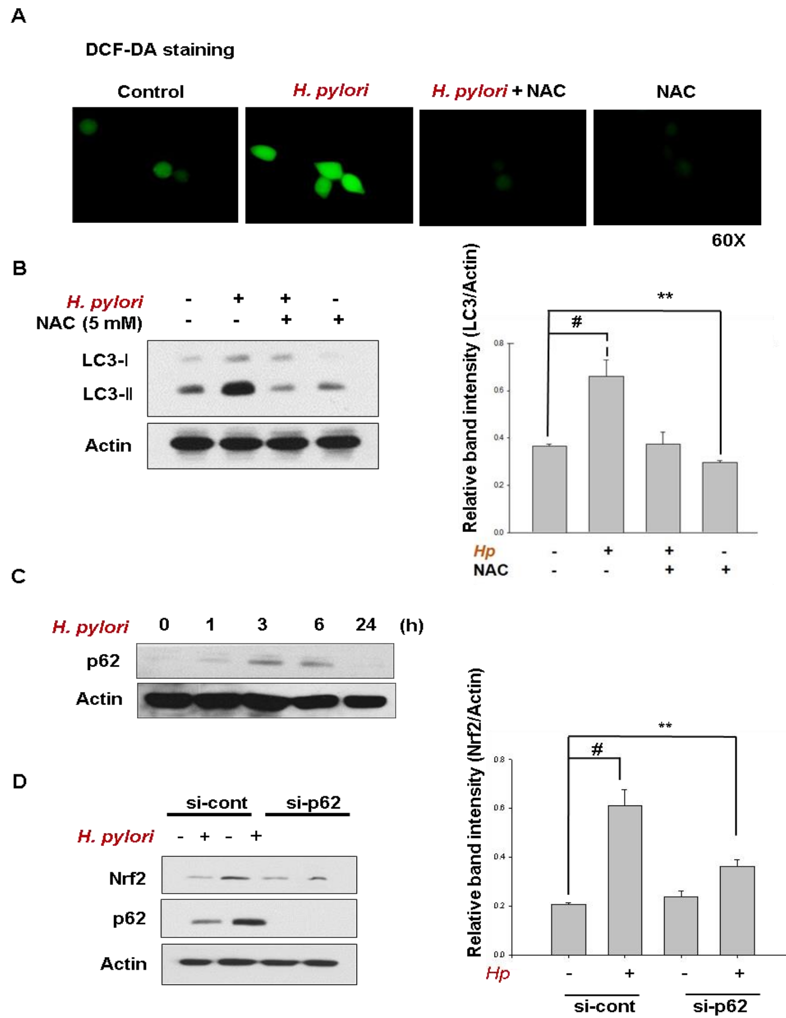


Figure 3. Involvement of ROS in *H. pylori*-induced LC3 expression in AGS cells and p62/SQSTM1 regulates the transcription factor Nrf2 activation

A. Intracellular ROS levels were determined based on the DCF-DA fluorescence.

Cells were incubated for 6 h with or without 5 mM NAC. Images were acquired by using a confocal laser-scanning microscope. **B**. *H. pylori*-induced LC3 expression was inhibited by treatment with NAC (5 mM) The expression level of LC3 is presented as means±S.D. *$P < 0.05$ **C**. Western blot analysis of p62/SQSTM1 expression. **D**. AGS cells were transiently transfected with specific small interfering RNA (siRNA) of p62/SQSTM1 using LipofectamineRNAiMAX followed by treatment with 100 MOI of *H. pylori* for 24 h. Nrf2 expression was abolished by p62/SQSTM1 siRNA.

Role of the Nrf2 target protein HO-1 in *H. pylori*-induced autophagy

The expression of HO-1, one of major target proteins of Nrf2, was increased after 12 h of incubation at translational (Fig. 4A) and transcriptional levels (Fig. 4B). When AGS cells were pre-treated with zinc protoporphyrin (ZnPP), a well-known inhibitor of HO-1 activity, 1 h before inoculating *H. pylori* for 24 h, the LC3 expression level was decreased dose-dependently (Fig. 4C). To prove that the increased HO-1 expression induced by *H. pylori* is responsible for promoting the autophagic process, AGS cells were infected with *H. pylori* in which the expression of HO-1 was knocked-down by transfecting with small interfering RNA (siRNA) of HO-1 Knock down of HO-1 abolished *H. pylori*-induced autophagy as evidenced by much lowered LC3 expression than the control (Fig. 4D).

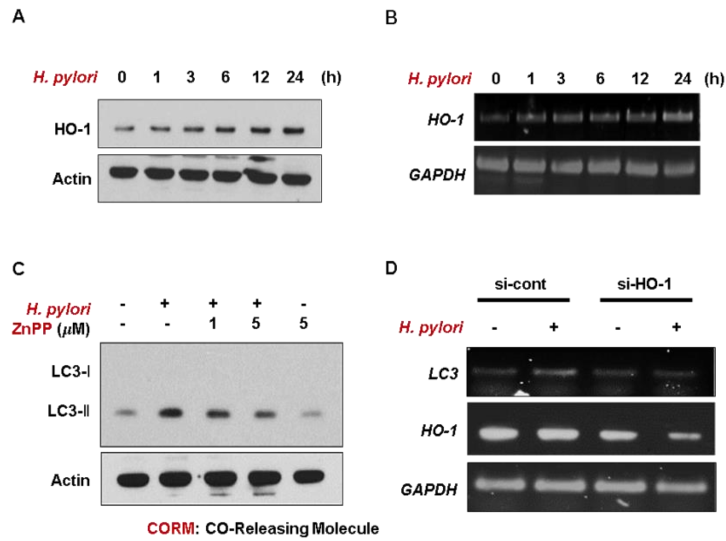


Figure 4. Role of the Nrf2 target protein HO-1 in *H. pylori*-induced autophagy in AGS cells

AGS cells were inoculated with the *H. pylori* ATCC43504 strain (100 MOI) for indicated time. **A**. HO-1 protein expression level **B**. HO-1 mRNA expression level **C**. Effect of ZnPP (HO-1 activity inhibitor) on *H. pylori*-induced LC3 expression **D**. AGS cells were transiently transfected with specific small interfering RNA (siRNA) of HO-1 using LipofectamineRNAiMAX and treated with 100 MOI of *H. pylori* for 24 h.

Carbon monoxide, a byproduct of HO-1, modulated *H. pylori*-induced autophagy.

CO exposure induced dose-dependent increases of LC3 expression in AGS cells (Fig. 5A). Expression of LC3 and Beclin1 was upregulated in a time-dependent manner (Fig. 5B). Another autophagy-related protein, Beclin1, is the mammalian ortholog of yeast ATG6 (Lei-lei Fu et al., 2013). LC3 and Beclin1 were also upregulated in a time-dependent manner (Fig. 5C). To check the autophagic cell death with FACS analysis, we used LysoTracker Red DND-99, which is a fluorescent acidotropic probe used for labeling and tracking acidic organelles in live cells. The FACS analysis demonstrated an increase of the fluorescence intensity of LysoTracker Red DND-99, which shows the effect of CORM on the induction of autophagic increase in AGS cells (Fig. 5D).

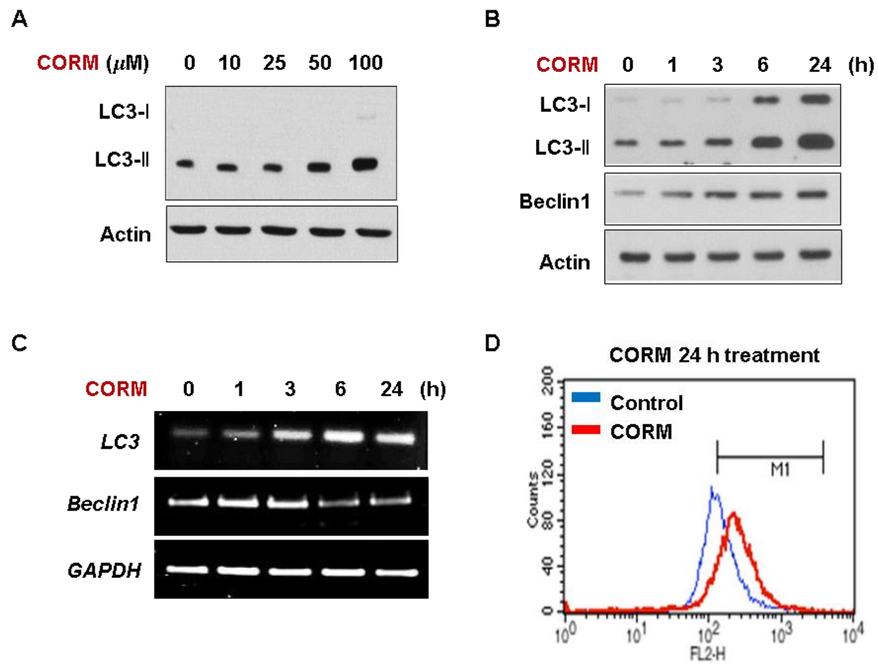


Figure 5. Role of CO, a byproduct of HO-1, in *H. pylori*-induced autophagy

A. LC3 was induced by treatment of CORM, which is a CO donor, in a dose-dependent manner. **B**. Autophagic proteins, LC3 and Beclin1 were upregulated with treatment of CORM (100 μM) **C**. The mRNA levels of LC3 and Beclin1 were increased in early time. **D**. AGS cells were pretreated with 100 μM CORM and incubated with 100 nM LysoTracker Red DND-99 for 30 min. FACS analysis demonstrated the increase of LysoTracker Red DND-99, which is an increase of autophagic death.

Potential link between autophagy and apoptosis

It has been indicated that autophagy may be cytoprotective, at least under conditions of nutrient depletion (Patricia Boya et al., 2005). To understand whether autophagy is involved in *H. pylori*-induced in AGS cell death, levels of some antiapoptotic proteins were measured. Interestingly, *H. pylori* infection upregulated anti-apoptotic proteins, while expression of the autophagic marker LC3 was increased (Fig. 6A). The ratio of pro- to anti-apoptotic protein Bax to Bcl-xL deacreased (Fig. 6B) CORM when treated to AGS cells, decreased the Bax/Bcl-xL ratio (Fig.6C and D).

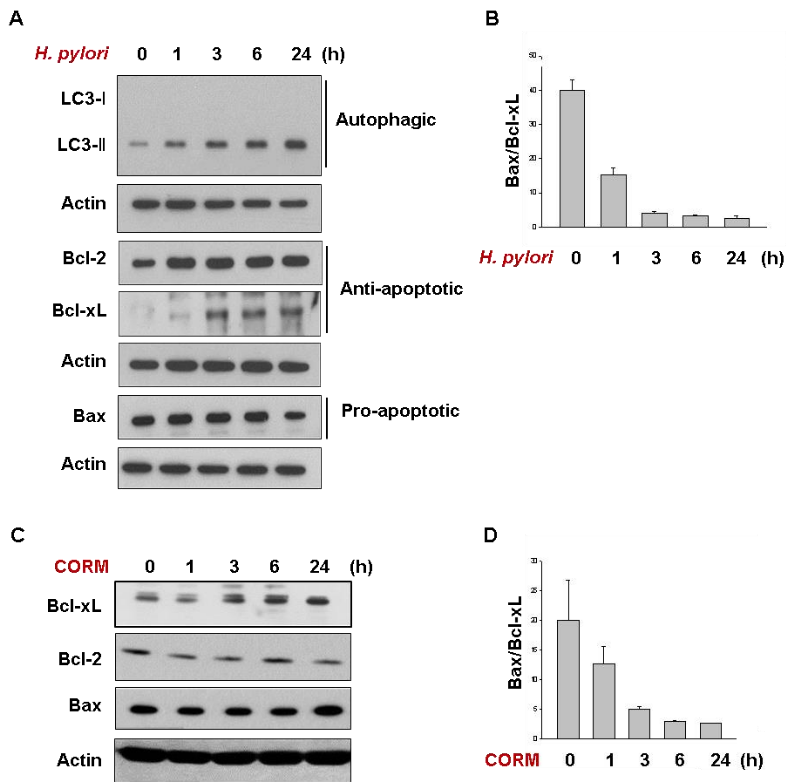


Figure 6. Potential link between autophagy and apoptosis

A. In AGS cells infected with *H. pylori*, anti-apoptotic proteins, Bcl-2 and Bcl-xL were upregulated. The level of pro-apoptotic protein Bax was not significantly changed. **B**. The ratio of Bax/Bcl-xL was decreased. **C** and **D**. Effects of CORM, on the Bax/Bcl-xL ratio.

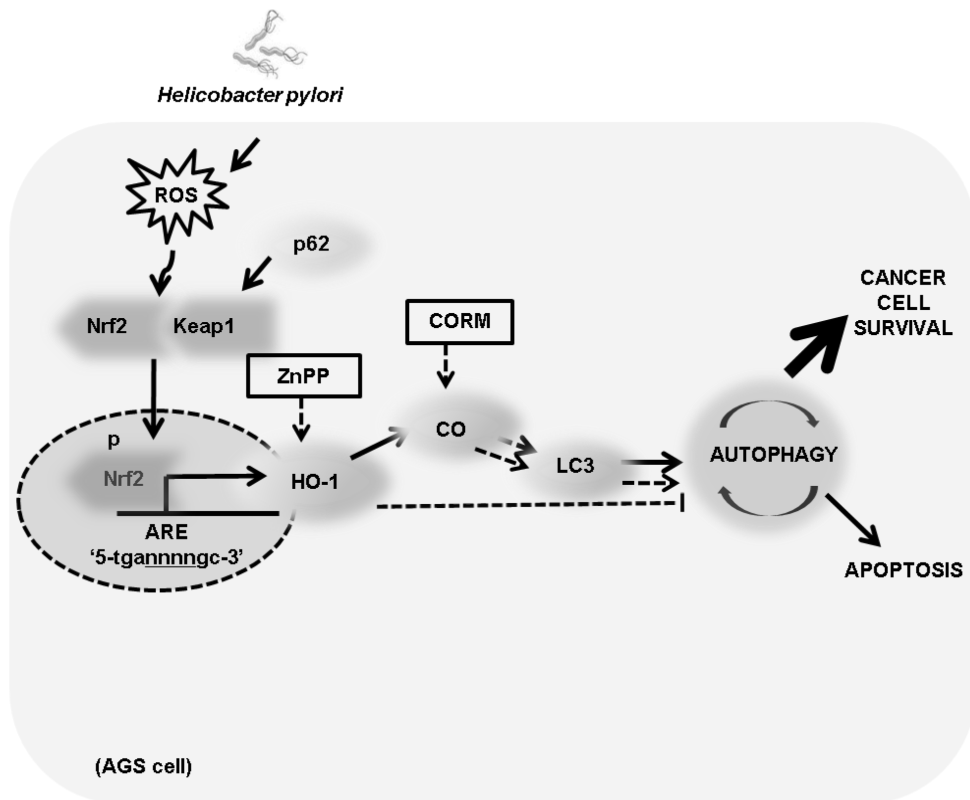
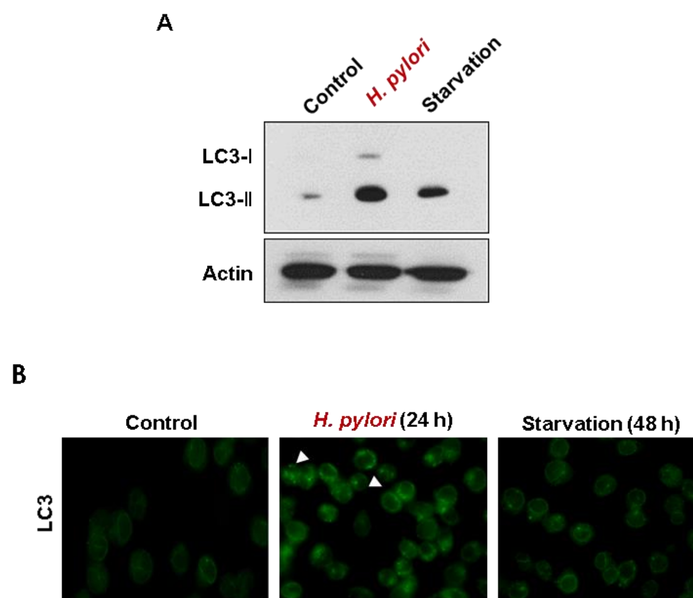


Figure 7. Schematic representation of the *H. pylori*-induced Nrf2 activation and HO-1 expression, leading to expression of the autophage marker LC3.

(Supplementary data)

H. pylori were infected into AGS cells for 24 h. To compare *H. pylori* group with a starvation group for 48 h as a positive control, I changed complete media to serum-free. The expression of LC3 and LC3 puncta was much highly upregulated in the *H. pylori* group compared with the starvation (Supplementary data A and B).



Supplementary data. *H. pylori* might be one of autophagic inducers

A. Western blot analysis **B**. Immunocytochemical analysis of LC3 protein expression induced by *H. pylori* and starvation for 48 h (positive control) in AGS cells

DISCUSSION

H. pylori infection has been regarded as an etiologic factor for several gastric diseases including gastric cancer. However, there are some unsolved questions about why the most of infected persons are asymptomatic, but others suffer from peptic ulcer disease, chronic gastritis, and cancer. One of papers published that only 2.1% of *H. pylori* infected individuals developed gastric cancer while the other 97.9% of individuals have mild gastritis or non-detectable symptom for 10 years of following study.

Autophagy is a self-degradative process that is important for balancing sources of energy at critical times in development and in response to nutrient stress (Danielle Glick et al., 2010). Among a variety of autophagic stimuli, I confirmed that *H. pylori* induced LC3 expression as meaning of induction of autophagy. Interestingly, I investigated that *H. pylori*-induced LC3 expression was much more highly upregulated than the starvation for 48 h group that is generally regarded as one of autophagic stimuli. Therefore, *H. pylori* may be one of strong autophagic inducers.

Among defence mechanisms, a regulatory mechanism known as the Nrf2/ARE signaling has evolved to induce phase II detoxifying or antioxidant enzymes, thereby conferring the protection of cellular DNA (Kwak et al., 2002). In the cytoplasm, Nrf2 is bound to and continuously degraded by its regulatory protein Keap1. Upon activation, Nrf2 translocates into the nucleus, whereby it binds to the

ARE promoter in cytoprotective genes such as HO-1 and NQO1 to regulate their expression. The Keap1-Nrf2 pathway is the major regulator of cytoprotective responses to endogenous and exogenous stresses caused by ROS and electrophiles. Interestingly, *H. pylori* induced Nrf2 to regulate autophagy. Moreover, *H. pylori* accumulated ROS which is involved in autophagy induction via Nrf2 activation.

To find out a novel mechanism in *H. pylori*-induced autophagy via Nrf2 activation, I checked the expression of p62. Notably, the transcription factor Nrf2 regulates the autophagic signaling pathway by modulating adaptor protein p62/SQSTM1. The selective autophagy substrate p62/SQSTM1 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1 (Komatsu M et al., 2010). As our expectation, *H. pylori*-induced autophagy also required p62. However, how p62/SQSTM1 would be influenced by ROS is still under investigation. Our study demonstrates that transcription factor Nrf2 is essential for the *H. pylori*-induced upregulation of HO-1 expression in AGS cells. In the present study, increased autophagic activity with upregulation of LC3 protein expression was accompanied by induction of HO-1. Furthermore, *H. pylori*-induced LC3 expression was mediated CO, a by-product of HO-1 which, in turn, modulates LC3 expression.

To reveal the significance of *H. pylori*-promoted autophagy, the levels of anti-apoptotic and pro-apoptotic proteins were measured. It has been reported that it is hard to precisely assess the association between autophagic and apoptotic process (Andrew Thorburn et al., 2008).

In conclusion, this study presents compelling evidence that *H. pylori* induces ROS production which, in turn, induce Nrf2 activation by p62/SQSTM1 As a result there is upregulated expression of HO-1, and its by-product carbon monoxide promotes autophagic process with suppression of apoptosis.

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국문 초록

위장상피세포에서 헬리코박터 파이로리에 의한 오토파지 유도에 있어서 Nrf2의 역할

헬리코박터 파이로리의 감염은 산화적 스트레스와 국소적 염증반응을 일으킴으로써 위염 및 위궤양, 더 나아가 위암으로 발전할 수 있는 것으로 알려져 있다. 전세계 인구의 50% 이상이 헬리코박터 파이로리에 감염되어 있다고 알려져 있지만, 80% 정도는 거의 병리학적 증상을 나타내지 않는다. 오토파지(autopahage)란 세포내 손상된 단백질이나 소기관을 제거하는 과정이며, 세포 내 항상성을 유지하 면서 종약억제나 종양세포 생존의 두 가지 역할을 한다고 알려져 있다. 하지만 헬리코박터 파이로리로 유도된 오토파지에 관한 메커니즘은 아직 잘알려져 있지 않다. 본 연구에서는 헬리코박터 파이로리 감염에 대한 체내 방어에 세포 내 다양 한 스트레스에 의해 활성화 되어 항산화, 해독화 효소들의 발현을 촉진시키는 전사인자로 알려진nuclear factor-erythroid 2p45 (NF-E2)-related factor (Nrf2)의 역할을 오토파지유도를 중심으로 살펴보았다

헬리코박터 파이로리를 처리시 활성산소의 세포내 축적과 Nrf2의 핵내

이동과 오토파지의 지표인 microtubule-associated light chain3 (LC3) 의 발현이 현저히 증가하였다. Nrf2가 LC3를 조절하는지를 알아보기 위해 본 연구자는 AGS 세포에 Nrf2 siRNA를 transfection하여 knockdown 시켰을 때, LC3의 발현량이 줄어 들었음을 확인하였으며, *nrf2* knockout mouse에서도 LC3의 발현량이 현저히 감소하었음을 확인 하였다. 또한 Nrf2의 활성과 그 단백질인 Hemeoxygenase-1 (HO-1) 의 단백질과 mRNA 발현 또한 증가함을 확인하고, HO-1이 LC3를 조절하는지를 확인해 보고자 하였다. HO-1 siRNA를 transfection 하여 knockdown에 의한 LC3 발현이 감소하였다. 한편, HO-1의 부산물인 일산화산소를 유리하는 화합물인 CO-releasing molecule (CORM) 처리시에는 오토파지와 관련된 LC3와 Beclin1의 발현량을 높임으로써 위장상피세포의 생존을 유도함을 확인할 수 있었다. 결론적으로, 헬리코박터 파이로리로 인해 생체 내 방어기전을 유도하는 Nrf2를 활성화 시킴으로써, 오토파지를 일으켜 위장상피세포 생존의 역할을 하는 것으로 사료된다

주요어 : 헬리코박터 파이로리, 오토파지, microtubule-associated light chain3 (LC3), Nrf2, Heme oxygenase-1 (HO-1), Carbon monoxide