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Original Article

High level of Helicobacter pylori infection contributes to the survival of colon cancer via up-regulating autophagy *in vitro*

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Abstract: Colon cancer (CRC) is one of the most prevalent gastrointestinal malignancies and the fourth leading cause of cancer death in the world. *Helicobacter pylori* (*H. pylori*) infection is prevalent in the population and is associated with kinds of digestive system diseases. In this study, we aimed to investigate the effect of *H. pylori* infection on the survival of colon cancer *in vitro*. Firstly, the expression of autophagy related protein-beclin1 was found aberrantly overexpressed in the tissue sample from colon cancer patients compared with the healthy control. Further research showed that cell apoptosis rate in SW480 cells was suppressed by *H. pylori* infection in a dose dependent manner. Western blot analysis showed that the expression of beclin1 was increased by *H. pylori* infection in dose dependent manner and the microtubule-associated protein 1 light chain 3 (LC3)-II/I ratio was obviously increased and the level of autophagy specific degradation protein p62 was decreased by *H. pylori* infection at 100 MOI. Moreover, high level of *H. pylori* infection promoted cell viability and suppressed cell apoptosis in SW480 cells. The expression of proliferation marker (Ki67) and apoptosis suppressing protein Bcl-2 was both up-regulated and the rate of cleaved-caspase-3/caspase-3 and the level of apoptosis promoting protein Bax was both suppressed in *H. pylori* infected SW480 cells compared with the control group. Moreover, autophagy inhibitor 3-MA counteracted the effect of *H. pylori* infection on cell viability. Taken together, we revealed that high level of *H. pylori* infection contributes to the survival of colon cancer *in vitro*. In consideration of the aberrant expression of beclin1, we speculated that beclin1 mediated the tumor-promoting activity of *H. pylori* infection.

Keywords: *Helicobacter pylori*, survival, colon cancer, beclin1

Introduction

Colon cancer (as named as colorectal cancer, CRC) is the third most prevalent gastrointestinal malignancy worldwide and the fourth leading cause of cancer death in the world, marked by highly metastatic and highly recurrent [1, 2]. As Doll and Peto suggested, as many as 90% of stomach and large bowel cancer-related deaths could be attributed to dietary factors [3]. Besides that, the metabolism of intestinal microflora is associated with colon cancer by activation of procarcinogens in the intestinal cavity [4]. Here, we aimed to investigate the effect of *Helicobacter pylori* (*H. pylori*) infection on the progression of colon cancer.

H. pylori is a spiral gram-negative, microaerobic bacterium and was first isolated from the gastric mucosa of gastritis patients by Marshall and Warren in 1983 [5]. *H. pylori* infection is prevalent in the population and is associated with kinds of digestive system diseases such as chronic gastritis (CG) [6], peptic ulcer (PU) [7] and gastric cancer [8]. *H. pylori* was classified as a group I carcinogen by the World Health Organization (WHO) in 1994 [9] and was etiologically responsible for over 50% of gastric cancer [10]. Neu B et al. reported that *H. pylori* infection induced the apoptosis of rat gastric parietal cells through the activation of NF-kappa B and induction of nitric oxide [11]. According to another research, the complex mechanisms of *H. pylori*-induced gastric carcinogenesis included disruption of the balance between cell proliferation and apoptosis, change in cell invasion and migration ability, induction of cell autopha-

gy [12]. In addition, recent studies have demonstrated the relationship between *H. pylori* infection and the risk of colon cancer [13]. However, the specific mechanisms of *H. pylori* infection in the development of colon cancer are still elusive.

Autophagy is an evolutionarily highly conserved process in eukaryotes, which plays a fundamental role in protein and organelle degradation and is closely involved in human disease and physiology [14]. Autophagy acts as a tumor suppressor or tumor promoter to regulate cancer cell proliferation or tumorigenesis in different model systems, indicating an important role of in cancers [15, 16]. Previous studies have also demonstrated the interrelation of autophagy with the progression of colon cancer [17, 18]. Beclin1 is highly homologous to the yeast autophagy gene Atg6/Vps30 and it is located at chromosomal 17q21 [19]. Beclin1 plays an essential role in the regulation of autophagosome formation and maturation [20]. Besides that, beclin1 plays a mediating effect on the information of autophagy and tumor development. According to the research of Wei et al, the up-regulated expression of phosphorylated beclin1 mutant leaded to reduced autophagy, thus promoting tumor growth and dedifferentiation in non-small cell lung carcinoma (NSCLC) [20]. Moreover, higher expression of beclin1 was found in CRC tissues compared with normal tissues and beclin1 served as a favorable prognostic marker in CRC diagnosis [21]. But till now, the effect of beclin1 on the survival of colon cancer has not been validated.

In this study, we aimed to investigate the effect of *H. pylori* infection on the survival of colon cancer *in vitro*. We hypothesized that high level of *H. pylori* infection contributes to the survival of CRC cells via up-regulating beclin1. Our results may provide a better understanding for the underlying pathological mechanism of CRC.

Materials and methods

Samples collection

The study was performed in accordance with the Helsinki Declaration and was approved by the ethics committee on human studies of Sichuan Provincial People's Hospital. Forty pairs of human colon cancer tissues and adjacent normal tissues were obtained from patients who underwent surgical resection in Sichuan Provincial People's Hospital. The patient was informed and the consents of the patients were obtained before the experiment. The tissues were frozen in liquid nitrogen and stored at -80°C until use.

RT-PCR

Total RNA was extracted from tumor tissues and using Trizol reagents (Invitrogen, USA) according to the manufacturer's instructions. Equal amounts of RNA were reversely transcribed to cDNA with HiScript Reverse Transcriptase Kit (Vazyme, China). PCR was performed with the following primers and standard profiles: Beclin1 (forward: 5'-GAACCGCAAGATAGTGGC-3'; reverse: 5'-CAGAGCATGGAGCAGCAA-3'). The annealing temperature and the thermal cycles for beclin1 were 54°C and 35 cycles, respectively. RT-PCR products were analyzed on 1% agarose gel and the intensity of each band was quantified by Image J software. The level of GAPDH was used as an internal standard.

Western blot analysis

The proteins extracted from tissues and cultured cells were separated on a 12% polyacrylamide gel and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Massachusetts, USA). The membranes were blocked in 1 × phosphate-buffered saline (PBS) containing 0.1% Tween 20 and 5% nonfat milk for 2 h at room temperature, and then were incubated with the primary antibodies: anti-beclin1, anti-microtubule-associated protein 1 light chain 3 (LC3)-I, anti-LC3-II, anti-p62, anti-Bcl-2, anti-Ki67, anti-caspase-3, anti-cleaved-caspase-3, anti-Bax, anti-GAPDH and corresponding HRP-conjugated secondary antibodies (Abcam, Cambridge, UK). Membranes were extensively washed 3-5 times with PBST. Proteins were detected using a ChemiDoc XRS imaging system and Quantity One analysis software (Bio-Rad, San Francisco, California, USA). GAPDH (Abcam) was used as an endogenous reference.

Cell culture

The human colon cancer cell line SW480 was purchased from American Type Culture

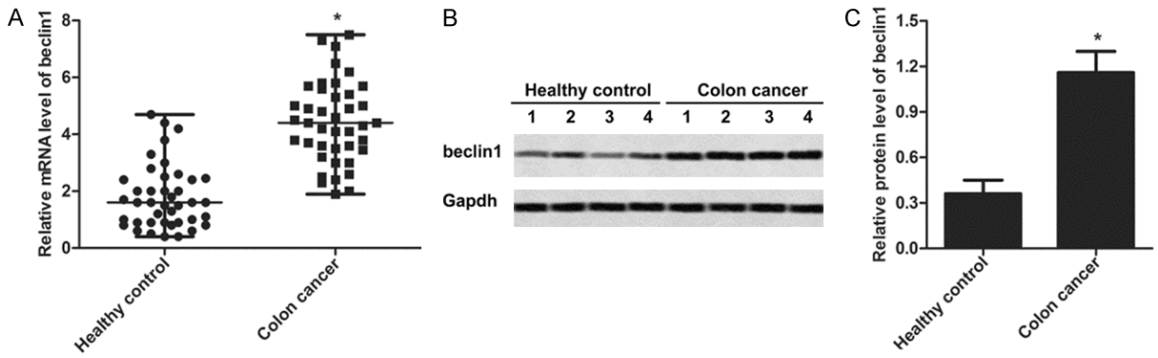

Figure 1. The expression of beclin1 was up-regulated in CRC. A. Relative mRNA level of beclin1 in CRC tissues and adjacent healthy tissues were detected through RT-PCR. B. Relative protein level of beclin1 in CRC tissues and adjacent healthy tissues was detected through western blotting. GAPDH was used as an endogenous reference. C. Histogram represents the statistical analysis of western blotting. The bars showed means ± SD of three independent experiments. $^{*}P < 0.05$ versus healthy control group.

Collection (Manassas, VA). The cell line was maintained routinely in RPMI Media 1640 (Gibco, Cat#:11875-093) supplemented with 10% fetal bovine serum (Life Technologies, Inc., Grand Island, New York) and grown at 37°C in humidified air containing 5% CO_2.

H. pylori strains culture and infection model

H. pylori strain SS1 (obtained from Chinese Centre for Disease Control and Prevention) was grown on brain-heart infusion plates containing 10% rabbit blood at 37°C under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2). *H. pylori* was washed from the culture plates with sterile phosphate-buffered saline (PBS) and was centrifuged at 2500 × g for 5 min, then was resuspended in RPMI 1640 medium without antibiotics. The amount of bacteria was determined by measuring optical density at 600 nm (1 OD_{600} = 1 × 10^9 CFU/ml). RPMI 1640 medium alone served as a blank control.

Drug

3-MA was purchased from Sigma (St. Louis, MO, USA) and was dissolved in sterile PBS to prepare 200 mM stock solution and aliquots were kept at -20°C.

Cell viability analysis

Cell viability was assessed using methylthiazoletetrazolium (MTT) assay. SW480 cells were seeded in 96-well plates (3 × 10^3 cells per well). Cells were incubated with 20 μl of MTT solution (5 mg/mL; Sigma) and 150 μl of Dimethyl sulfoxide (DMSO) for 4 hours at 37°C. OD value was determined with a microplate spectrophotometer (ELx800; Bio-TEK, Win ooski, Vermont) at the indicated time points after treatment at 490 nm wavelengths.

Flow cytometric analysis of cell apoptosis

For the apoptosis analysis, cells were collected, washed twice with cold PBS, re-suspended and fixed, then were stained using the Annexin V-fluorescein isothiocyanate (FITC) and PI apoptosis detection kits (Annexin V-FITC Apoptosis Detection Kit, eBioscience). The cells were examined by the FACS Caliber II sorter and Cell Quest FACS system (BD Bio-sciences, San Jose, CA, USA) according to the manufacturer's protocols.

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 6.0 software (San Diego, CA, USA). Unpaired Student's t test was used to compare two groups, and one-way ANOVA was used to compare three or more groups. $P < 0.05$ deemed as statistically significant.

Results

Expression of beclin1 was up-regulated in CRC

Autophagy related gene beclin1 is usually found down-regulated in cancers but it has also been reported that the expression of beclin1 is aber-

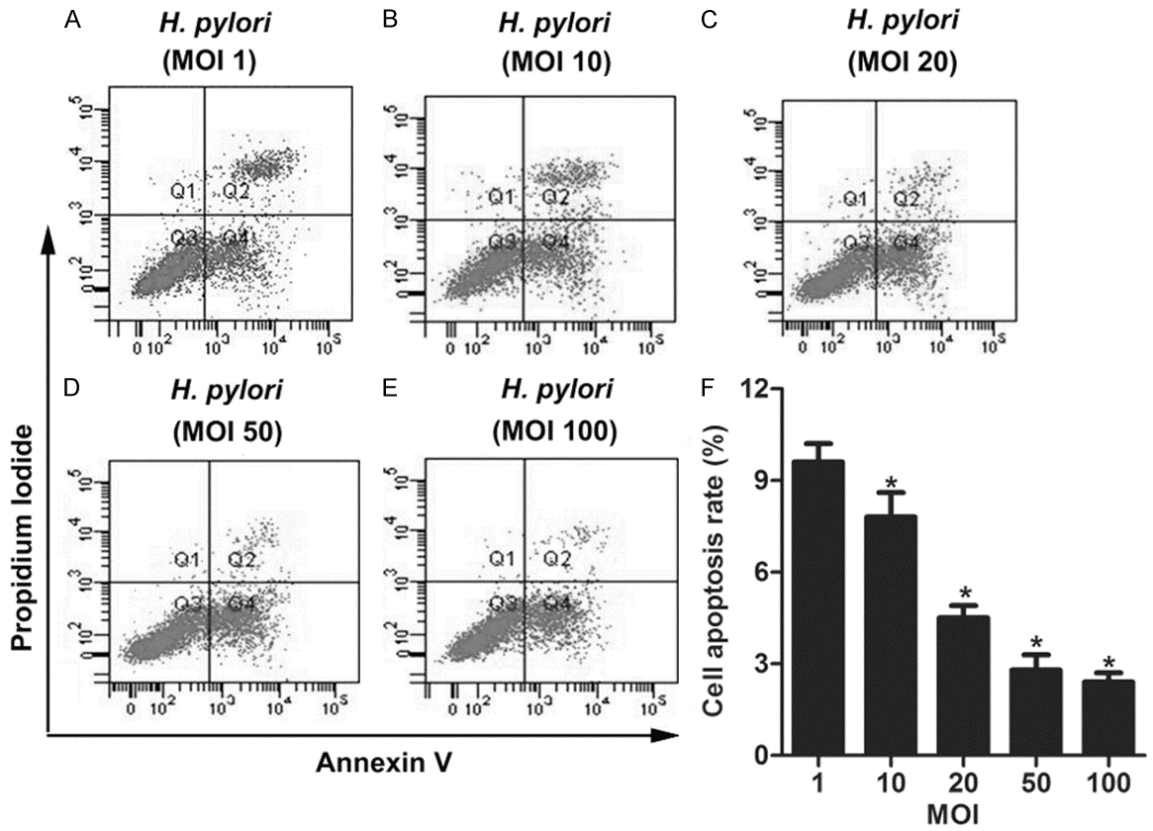

Figure 2. Cell apoptosis rate in SW480 cell line was suppressed by *H. pylori* infection in a dose dependent manner. A-E. SW480 cells were infected with *H. pylori* at different MOI range from 1 to 100, respectively. Flow cytometry was performed to determine the percentages of apoptosis in SW480 cells. F. Histogram represents the statistical analysis of flow cytometry. The bars showed means ± SD of three independent experiments. $^{*}P < 0.05$ versus MOI = 1 group.

rant in CRC [22]. So the expression of beclin1 in serum sample from CRC patients and healthy control was detected through RT-PCR and western blotting, respectively. As shown in **Figure 1A**, relative mRNA level of beclin1 was significantly higher in CRC group than the healthy control group. Western blot analysis further showed that relative protein level of beclin1 was also up-regulated in CRC group compared with the healthy control group ($^{*}P < 0.05$, **Figure 1B**, **1C**). These results suggested that the expression of beclin1 was aberrantly up-regulated in CRC.

Cell apoptosis rate in SW480 cell line was suppressed by H. pylori infection in a dose dependent manner

SW480 cells were infected with *H. pylori* at different multiplicity of infection (MOI). As shown in **Figure 2A-E**, cell apoptosis rate tended to decrease with the gradually increased MOI from 1 to 100. Statistical results of apoptosis rate in SW480 cells at different MOI was presented in the form of histogram ($^{*}P < 0.05$, **Figure 2F**). These results above suggested that cell apoptosis rate in SW480 cells was suppressed by *H. pylori* infection in a dose dependent manner.

H. pylori infection induced autophagy in CRC

Having known that *H. pylori* infection and beclin1 were both involved in the progression of CRC, the underlying correlation between the two was further investigated. The expression of autophagy related proteins beclin1, LC3-I, LC3-II, p62 in pre-treated SW480 cells was detected through western blotting. As shown in **Figure 3A**, **3B**, the expression of beclin1 was increased by *H. pylori* infection in dose dependent manner. Further investigation found that LC3-II/I ratio was also increased by *H. pylori* infection. At the same time, the expression level of

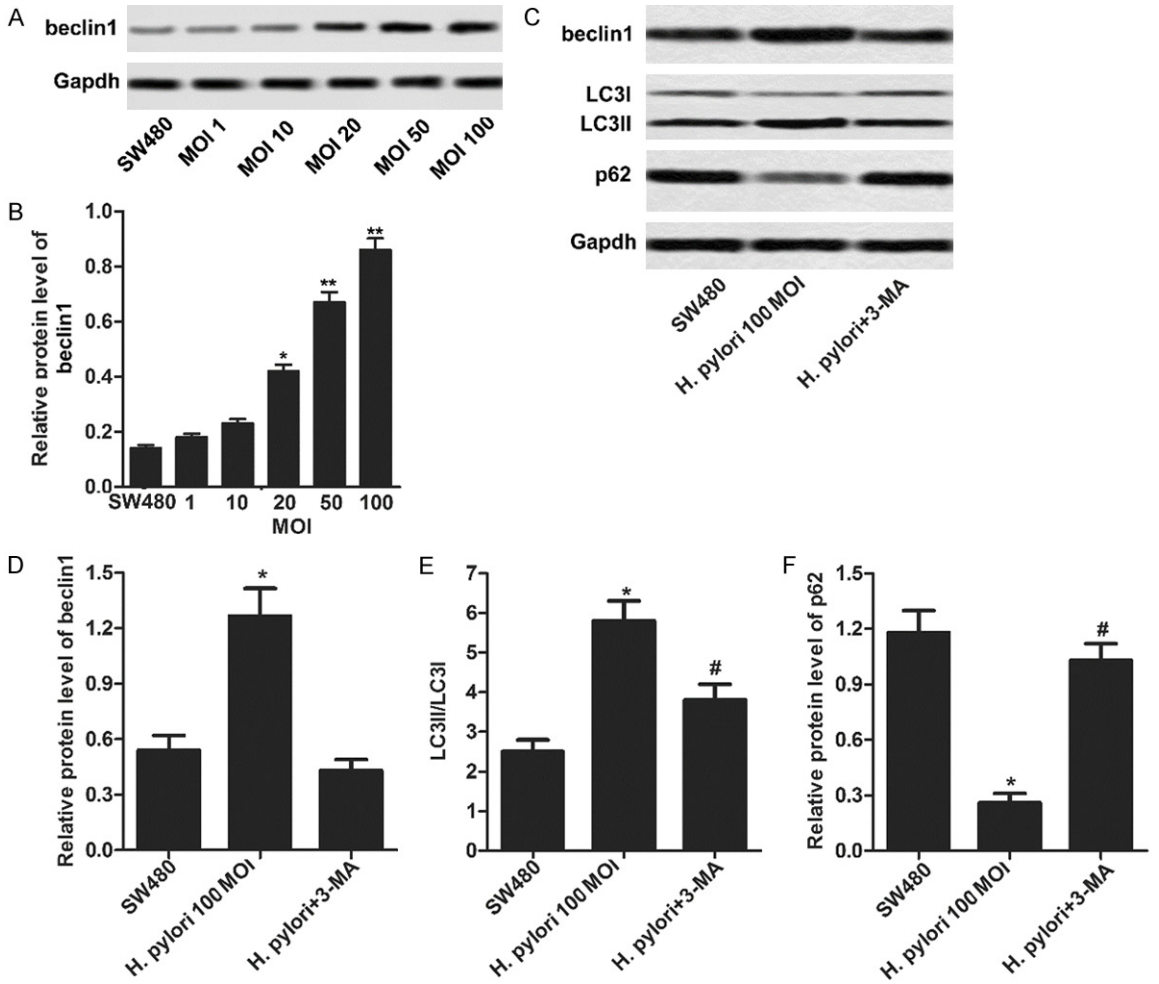

Figure 3. *H. pylori* infection induced autophagy in CRC. A. SW480 cells were infected with H. pylori at different MOI range from 1 to 100, respectively. The effect of H. pylori infection on the expression of Beclin1 was valued through western blotting. B. Histogram represents the statistical analysis of beclin1 expression. In the following experiments, SW480 cells were divided into 3 groups: Untreated SW480 cells were used as control group. SW480 cells infected with H. pylori at 100 MOI alone or in combination with 3-MA (5 mM) for 72 h were used as experiment groups. C. The expression of autophagy related proteins beclin1, LC3-I/II, p62 was detected through western blotting. GAPDH was used as an endogenous reference. D. Histogram represents the statistical analysis of beclin1 expression detected through western blotting. E. Histogram represents the statistical analysis of LC3-II/I ratio detected through western blotting. F. Histogram represents the statistical analysis of p62 detected through western blotting. The bars showed means ± SD of three independent experiments. $^{*}P < 0.05$ versus SW480 control group, $^{**}P < 0.01$ versus SW480 control group, $^{\#}P < 0.05$ versus *H. pylori* infected SW480 group.

autophagy specific degradation protein p62 was suppressed by *H. pylori* infection. Moreover, the addition of autophagy inhibitor 3-MA counteracted the inducing effect of *H. pylori* infection on autophagy in SW480 cells. The expression of beclin1, p62 and the LC3-II/I ratio detected through western blotting was statistically analyzed and was presented in the form of histogram ($^{\#}P < 0.05$, $^{*}P < 0.05$, **Figure 3D-F**). These results above indicated that *H. pylori* infection induced autophagy in CRC.

H. pylori infection induced cell proliferation was counteracted by 3-MA treatment in CRC

To investigate the molecular mechanisms of *H. pylori* infection on CRC cell survival, MTT assay was conducted to detect cell proliferation in SW480 cells. As shown in **Figure 4A**, relative cell proliferation rate was increased by *H. pylori* infection in SW480 cells compared with the control cells. Then elevated cell proliferation rate was suppressed by the addition of 3-MA ($^{\#}P < 0.05$, $^{*}P < 0.05$). The expression of cell

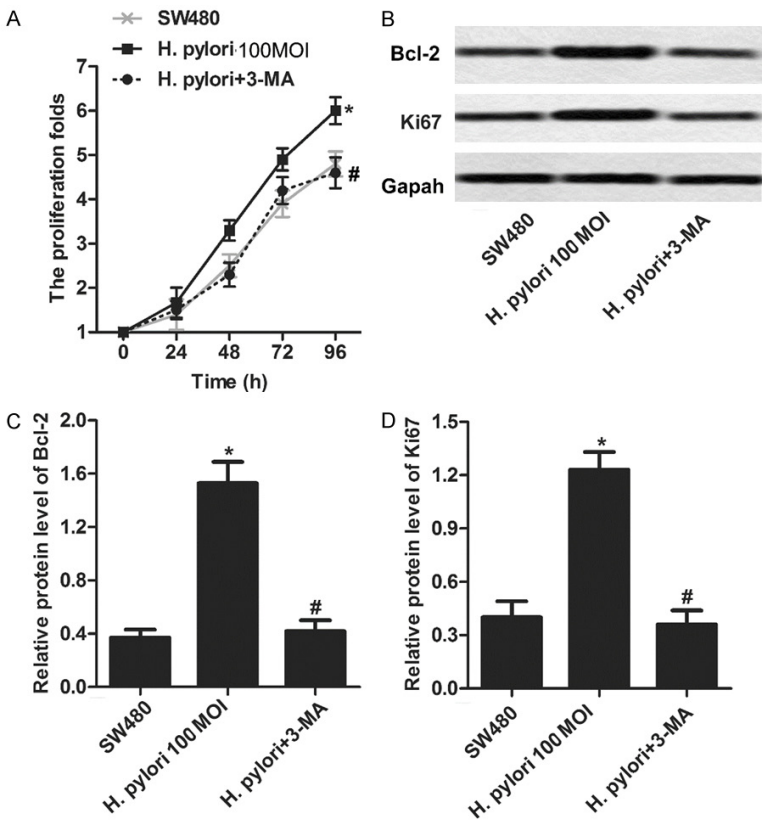

Figure 4. *H. pylori* infection induced cell proliferation was counteracted by 3-MA treatment in CRC. A. Cell proliferation rate of SW480 cells in different group was detected through MTT assay at indicated times. B. The expression of cell proliferation marker protein Ki67 and apoptosis suppressing protein Bcl-2 in SW480 cells was detected through western blotting. GAPDH was used as an endogenous reference. C and D. Histograms represent the statistical analysis of Bcl-2 and Ki67 detected through western blotting, respectively. The bars showed means ± SD of three independent experiments. $^{*}P < 0.05$ versus SW480 control group. $^{\#}P < 0.05$ versus *H. pylori* infected SW480 group.

proliferation marker protein Ki67 and apoptosis suppressing protein Bcl-2 was both increased in *H. pylori*-infected SW480 cells. But, 3-MA counteracted the facilitating effect of *H. pylori* infection on the expression of Ki67 and Bcl-2 (**Figure 4B**). The expression of Bcl-2 and Ki67 detected through western blotting was statistically analyzed and was presented in the form of histogram, respectively ($^{\#}P < 0.05$, $^{*}P < 0.05$, **Figure 4B**, **4C**). These results above indicated that *H. pylori* infection induced cell proliferation was counteracted by 3-MA treatment in CRC

Inhibiting effect of H. pylori infection on cell apoptosis was counteracted by 3-MA treatment in CRC

To further explore the mechanism of *H. pylori* infection on CRC cell apoptosis, flow cytometric analysis was conducted to detect cell apoptosis in SW-480 cells. Cell apoptosis rate was largely suppressed by *H. pylori* infection compared with the control cells. Suppressed cell apoptosis rate was then elevated by 3-MA in the *H. pylori*+ 3-MA group compared with the H. pylori infection group ($^{\#}P < 0.05$, $^{*}P < 0.05$, **Figure 5A**, **5B**). The expression of apoptosis marker protein cleaved-caspase-3/caspase-3 and apoptosis promoting protein Bax was also detected through western blotting. Western blotting analysis showed that the rate of cleaved-caspase-3/caspase-3 and the expression level of Bax were both down-regulated in the *H. pylori* infection group. Suppressed expression of cleaved-caspase-3 and Bax was increased by adding 3-MA in the *H. pylori*+ 3-MA group (**Figure 5C**). The rate of cleaved-caspase-3/caspase-3 and the level of Bax detected through western blotting was statistically analyzed and was presented in the form of histogram ($^{\#}P < 0.05$, $^{*}P < 0.05$, **Figure 5D**, **5E**). These results above indicated that the inhibiting effect of *H. pylori* infection on cell apoptosis was counteracted by 3-MA treatment in CRC.

Discussion

Colon cancer (CRC) is one of the most common cancers and is one of the leading causes of cancer-related deaths in the world. *H. pylori* infection is highly associated with gastrointestinal diseases, including gastric inflammation, gastric cancer, peptic ulcer and gastric mucosa-associated lymphoid-tissue lymphoma. In the present study, we aimed to investigate the effect of *H. pylori* infection on the survival of colon cancer.

H. pylori infection relies on an acid acclimation mechanism for its survival in the acidic stomach, and then adsorption to host cells by flagellar motility, thus causing tissue damage by

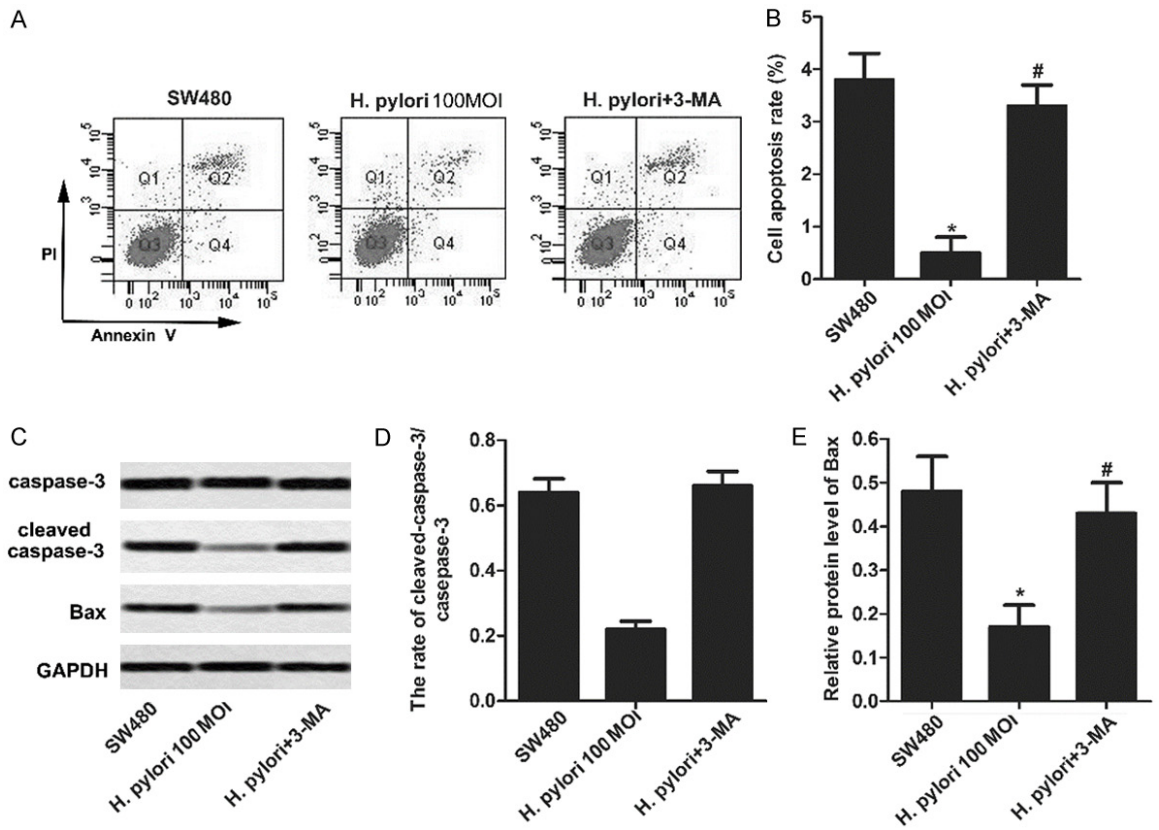

Figure 5. The inhibiting effect of *H. pylori* infection on cell apoptosis was counteracted by 3-MA treatment in CRC. A. Flow cytometric analysis was conducted to detect cell apoptosis in SW480 cells. B. Histograms represent the statistical analysis of flow cytometric. C. The expression of apoptosis marker protein cleaved-caspase-3 and apoptosis promoting protein Bax was detected through western blotting. GAPDH was used as an endogenous reference. D and E. Histograms represent the statistical analysis of cleaved-caspase-3 and Bax detected through western blotting, respectively. The bars showed means ± SD of three independent experiments. $^{*}P < 0.05$ versus SW480 control group. $^{\#}P < 0.05$ versus *H. pylori* infected SW480 group.

toxin releasing [23]. Cytotoxin-associated gene A (Cag A), vacuolating cytotoxin A (VacA) and peptidoglycan are most studied virulence factors in the infection progression of *H. pylori.* Cag A regulated the proliferation and trans-differentiation of gastric epithelial cells multiple signaling pathways [24, 25]. Others have demonstrated the pro-apoptosis effect of VacA in human gastric epithelial cells by targeting mitochondria [26]. Peptidoglycan was also reported to decrease the threshold for malignant transformation by suppressing apoptosis and promoting proliferation and cell migration [27, 28]. Moreover, autophagy was reported to be induced by VacA in human-derived gastric epithelial cells (AGS) for limiting the toxin-induced cellular damage [29]. At the same time, prolonged exposure to VacA suppressed autophagy by preventing maturation of the autolysosome to facilitate intracellular survival and persistence of *H. pylori* [30]. The previous studies reveal that autophagy is closely associated with *H. pylori* infection. The formation and suppression of autophagy induced by *H. pylori* infection are always in the state of dynamic balance.

Beclin1 is an essential molecule in the formation of autophagosome, which involves in the formation and maturation of autophagosome by mediating the binding of autophagy related proteins to phagophore [31]. In previous reports, beclin1 usually acted as an important tumor suppressor gene in carcinogenic process and the expression of beclin1 is usually found down-regulated in cancers. For example, the expression levels of beclin1 mRNA and protein were significantly reduced in the NSCLC tissues compared with the matched surrounding normal lung tissues [32]. Overexpressed beclin1

arrested the cell cycle, suppressed cell proliferation, promoted autophagy and apoptosis in ovarian cancer and was associated with a lower risk of death in patients with ovarian cancer [33]. But, as Wu S et al. reported, aberrant elevated expression of autophagy related proteins (beclin1, LC3 and mTOR) may be associated with the development and progression of CRC [22]. Consistent with this report, in our study, the expression of beclin1 in mRNA and protein level was both largely decreased compared with the healthy control. Thus, the aberrant expression of beclin1 in CRC indicates a certain relationship with CRC progression.

According to previous reports, *H. pylori* infection was closely associated with cell apoptosis. For example, overexpressed tumor necrosis factor receptor-associated factor 1 (TRAF1) induced by *H. pylori* infection plays an anti-apoptotic role in gastric epithelial cells and may contributes to the gastric carcinogenesis [34]. Besides that, Cag A promoted host cell survival and suppressed cell apoptosis to overcome self-renewal of the gastric epithelium, thus enhancing the colonization of *H. pylori.* in the stomach [35]. Also, in our study, cell apoptosis rate in SW480 cell line was suppressed by *H. pylori* infection in a dose dependent manner. In the study of autophagy, beclin1, LC3 and p62/SQSTM1 (p62) are three important autophagy-related proteins [36]. In our study, the expression of beclin1 and the LC3-II/I ratio were significantly increased by *H. pylori* infection, and at the same time, the expression of autophagy specific degradation protein p62 was decreased. As expect, autophagy inhibitor 3-MA suppressed the formation of autophagy induced by *H. pylori* infection. The results above strongly suggest that *H. pylori* infection was associated with CRC progression by inducing autophagy.

In previous researches, autophagy was reported to be associated with cell proliferation in kinds of diseases. For example, down-regulated MEG3 activated autophagy and increased cell proliferation in bladder cancer [37]. Others reported that overexpressed BRAF-activated lncRNA (BANCR) induced an increase in the ratio of LC3-II/I and promoted cell proliferation in papillary thyroid carcinoma (PTC) [38]. Similarly, in our study, cell proliferation was obviously promoted in SW480 cells infected with *H. pylori* compared with the control cells. The expression of cell proliferation marker protein Ki67 and apoptosis suppressing protein Bcl-2 was both increased in *H. pylori*-infected SW480 cells. The addition of 3-MA counteracted the facilitating effect of *H. pylori* infection on cell proliferation. The results above identify that *H. pylori* infection promoted cell proliferation by activating autophagy in CRC.

Recent studies have revealed the possible crosstalk between *H. pylori* infection/autophagy and apoptosis. As reported, Bcl-2 and Bcl-xL appear to inhibit beclin1-mediated autophagy by binding to beclin1 [39]. Autophagy inhibitor 3-methyladenine (3-MA) and small interfering RNA (siRNA) targeting beclin1 suppressed cell apoptosis in human hepatocellular carcinoma cells [40]. Moreover, *H. pylori* infection was reported to regulate cell apoptosis in human gastric epithelial cells [41], peripheral blood monocytes [42]. In agreement with these reports, in our study, cell apoptosis was suppressed by *H. pylori* infection in SW480 cells compared with the control cells. The suppressed cell apoptosis rate was then elevated by 3-MA in *H. pylori*-infected SW480 cells. Moreover, the decreased expression of apoptosis marker protein cleaved-caspase-3 and apoptosis promoting protein Bax was up-regulated by adding 3-MA in *H. pylori*-infected SW480 cells. The results above identify that *H. pylori* infection suppressed cell apoptosis via autophagy in CRC.

In conclusion, this study demonstrated that the expression of beclin1 was aberrantly up-regulated in colon cancer. Besides that, cell apoptosis rate in SW480 cell line was suppressed by *H. pylori* infection in a dose dependent manner. At the same time, high level of *H. pylori* infection induced autophagy in CRC. Moreover, *H. pylori* infection promoted cell proliferation and suppressed cell apoptosis in CRC by inducing autophagy. So we conjectured that high level of *H. pylori* infection contributes to the survival of CRC via up-regulating beclin1.

Disclosure of conflict of interest

None.

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References

[1] Tenesa A and Dunlop MG. New insights into the aetiology of colorectal cancer from genome-wide association studies. Nat Rev Genet 2009; 10: 353-358.

[2] Pugh SA, Shinkins B, Fuller A, Mellor J, Mant D and Primrose JN. Site and stage of colorectal cancer influence the likelihood and distribution of disease recurrence and postrecurrence survival: data from the FACS randomized controlled trial. Ann Surg 2016; 263: 1143-1147.

[3] Doll R and Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 1981; 66: 1191-1308.

[4] Gorbach SL. The intestinal microflora and its colon cancer connection. Infection 1982; 10: 379-384.

[5] Warren JR and Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; 1: 1273-1275.

[6] Inoue I, Kato J, Tamai H, Iguchi M, Maekita T, Yoshimura N and Ichinose M. Helicobacter pylori-related chronic gastritis as a risk factor for colonic neoplasms. World J Gastroenterol 2014; 20: 1485-1492.

[7] Fashner J and Gitu AC. Diagnosis and Treatment of Peptic Ulcer Disease and H. pylori Infection. Am Fam Physician 2015; 91: 236-242.

[8] Wang F, Meng W, Wang B and Qiao L. Helicobacter pylori-induced gastric inflammation and gastric cancer. Cancer Lett 2014; 345: 196-202.

[9] Infection with Helicobacter pylori. IARC Monogr Eval Carcinog Risks Hum 1994; 61: 177-240.

[10] Covacci A, Telford JL, Del Giudice G, Parsonnet J and Rappuoli R. Helicobacter pylori virulence and genetic geography. Science 1999; 284: 1328-1333.

[11] Neu B, Randlkofer P, Neuhofer M, Voland P, Mayerhofer A, Gerhard M, Schepp W and Prinz C. Helicobacter pylori induces apoptosis of rat gastric parietal cells. Am J Physiol Gastrointest Liver Physiol 2002; 283: G309-318.

[12] Meng W, Bai B, Sheng L, Li Y, Yue P, Li X and Qiao L. Role of Helicobacter pylori in gastric cancer: advances and controversies. Discov Med 2015; 20: 285-293.

[13] Zhao YS, Wang F, Chang D, Han B and You DY. Meta-analysis of different test indicators: helicobacter pylori infection and the risk of colorectal cancer. Int J Colorectal Dis 2008; 23: 875-882.

[14] Mizushima N and Komatsu M. Autophagy: renovation of cells and tissues. Cell 2011; 147: 728-741.

[15] Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, Ohsumi Y, Cattoretti G and Levine B. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003; 112: 1809-1820.

[16] Yang S, Wang X, Contino G, Liesa M, Sahin E, Ying H, Bause A, Li Y, Stommel JM, Dell'antonio G, Mautner J, Tonon G, Haigis M, Shirihai OS, Doglioni C, Bardeesy N and Kimmelman AC. Pancreatic cancers require autophagy for tumor growth. Genes Dev 2011; 25: 717-729.

[17] Zhai H, Song B, Xu X, Zhu W and Ju J. Inhibition of autophagy and tumor growth in colon cancer by miR-502. Oncogene 2013; 32: 1570-1579.

[18] Coker-Gurkan A, Arisan ED, Obakan P, Guvenir E and Unsal NP. Inhibition of autophagy by 3-MA potentiates purvalanol-induced apoptosis in Bax deficient HCT 116 colon cancer cells. Exp Cell Res 2014; 328: 87-98.

[19] Cao Y and Klionsky DJ. Physiological functions of Atg6/Beclin 1: a unique autophagy-related protein. Cell Res 2007; 17: 839-849.

[20] Kihara A, Kabeya Y, Ohsumi Y and Yoshimori T. Beclin-phosphatidylinositol 3-kinase complex functions at the trans-Golgi network. EMBO Rep 2001; 2: 330-335.

[21] Yang Z, Ghoorun RA, Fan X, Wu P, Bai Y, Li J, Chen H, Wang L and Wang J. High expression of Beclin-1 predicts favorable prognosis for patients with colorectal cancer. Clin Res Hepatol Gastroenterol 2015; 39: 98-106.

[22] Wu S, Sun C, Tian D, Li Y, Gao X, He S and Li T. Expression and clinical significances of Beclin1, LC3 and mTOR in colorectal cancer. Int J Clin Exp Pathol 2015; 8: 3882-3891.

[23] Cid TP, Fernandez MC, Benito Martinez S and Jones NL. Pathogenesis of Helicobacter pylori infection. Helicobacter 2013; 18 Suppl 1: 12-17.

[24] Murata-Kamiya N, Kurashima Y, Teishikata Y, Yamahashi Y, Saito Y, Higashi H, Aburatani H, Akiyama T, Peek RM Jr, Azuma T and Hatakeyama M. Helicobacter pylori CagA interacts with E-cadherin and deregulates the beta-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. Oncogene 2007; 26: 4617-4626.

[25] Suzuki M, Mimuro H, Kiga K, Fukumatsu M, Ishijima N, Morikawa H, Nagai S, Koyasu S, Gilman RH, Kersulyte D, Berg DE and Sasakawa C. Helicobacter pylori CagA phosphorylation-independent function in epithelial proliferation

and inflammation. Cell Host Microbe 2009; 5: 23-34.
[26] Chiozzi V, Mazzini G, Oldani A, Sciullo A, Ventura U, Romano M, Boquet P and Ricci V. Relationship between Vac A toxin and ammonia in Helicobacter pylori-induced apoptosis in human gastric epithelial cells. J Physiol Pharmacol 2009; 60: 23-30.
[27] Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Memet S, Huerre MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philpott DJ and Ferrero RL. Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. Nat Immunol 2004; 5: 1166-1174.
[28] Watanabe T, Asano N, Fichtner-Feigl S, Gorelick PL, Tsuji Y, Matsumoto Y, Chiba T, Fuss IJ, Kitani A and Strober W. NOD1 contributes to mouse host defense against Helicobacter pylori via induction of type I IFN and activation of the ISGF3 signaling pathway. J Clin Invest 2010; 120: 1645-1662.
[29] Terebiznik MR, Raju D, Vazquez CL, Torbricki K, Kulkarni R, Blanke SR, Yoshimori T, Colombo MI and Jones NL. Effect of Helicobacter pylori's vacuolating cytotoxin on the autophagy pathway in gastric epithelial cells. Autophagy 2009; 5: 370-379.
[30] Greenfield LK and Jones NL. Modulation of autophagy by Helicobacter pylori and its role in gastric carcinogenesis. Trends Microbiol 2013; 21: 602-612.
[31] Shimizu T, Marusawa H, Watanabe N and Chiba T. Molecular Pathogenesis of Helicobacter pylori-Related Gastric Cancer. Gastroenterol Clin North Am 2015; 44: 625-638.
[32] Jiang L, Liang X, Liu M, Wang W, Ma J, Guo Q, Han L, Yang C and Nan K. Reduced expression of liver kinase B1 and Beclin1 is associated with the poor survival of patients with non-small cell lung cancer. Oncol Rep 2014; 32: 1931-1938.
[33] Cai M, Hu Z, Liu J, Gao J, Liu C, Liu D, Tan M, Zhang D and Lin B. Beclin 1 expression in ovarian tissues and its effects on ovarian cancer prognosis. Int J Mol Sci 2014; 15: 5292-5303.
[34] Wan XK, Yuan SL, Tao HX, Diao LP, Wang YC, Cao C and Liu CJ. The Upregulation of TRAF1 Induced by Helicobacter pylori plays an anti-apoptotic effect on the infected cells. Helicobacter 2016; 21: 554-564.
[35] Mimuro H, Suzuki T, Nagai S, Rieder G, Suzuki M, Nagai T, Fujita Y, Nagamatsu K, Ishijima N, Koyasu S, Haas R and Sasakawa C. Helicobacter pylori dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. Cell Host Microbe 2007; 2: 250-263.
[36] Zheng S, Han F, Shi Y, Wen L and Han D. Single-prolonged-stress-induced changes in autophagy-related proteins Beclin-1, LC3, and p62 in the medial prefrontal cortex of rats with post-traumatic stress disorder. J Mol Neurosci 2017;
[37] Ying L, Huang Y, Chen H, Wang Y, Xia L, Chen Y, Liu Y and Qiu F. Downregulated MEG3 activates autophagy and increases cell proliferation in bladder cancer. Mol Biosyst 2013; 9: 407-411.
[38] Wang Y, Guo Q, Zhao Y, Chen J, Wang S, Hu J and Sun Y. BRAF-activated long non-coding RNA contributes to cell proliferation and activates autophagy in papillary thyroid carcinoma. Oncol Lett 2014; 8: 1947-1952.
[39] Zhou F, Yang Y and Xing D. Bcl-2 and Bcl-xL play important roles in the crosstalk between autophagy and apoptosis. FEBS J 2011; 278: 403-413.
[40] Wang Y, Han C, Lu L, Magliato S and Wu T. Hedgehog signaling pathway regulates autophagy in human hepatocellular carcinoma cells. Hepatology 2013; 58: 995-1010.
[41] Xie J, Lin Z, Xian Y, Kong S, Lai Z, Ip S, Chen H, Guo H, Su Z, Yang X, Xu Y and Su Z. (-)-Patchouli alcohol protects against Helicobacter pylori urease-induced apoptosis, oxidative stress and inflammatory response in human gastric epithelial cells. Int Immunopharmacol 2016; 35: 43-52.
[42] Zhang Y, Sun H, Zhao H, Chen X, Li J and Li B. Early apoptosis of monocytes induced by Helicobacter pylori infection through multiple pathways. Dev Comp Immunol 2017; 73: 46-51.