Revista Argentina de Clínica Psicológica
2020, Vol. XXIX, N°4, 872-876
DOI: 10.24205/03276716.2020.701

Helicobacter Pylori Regulates the Autophagy and Apoptosis Of CACO-2 Cells Through the Nrf2 Signaling Pathway

Chun Yang[a],ShaoPing Deng[b*]

Abstract

To study the mechanism of Helicobacter pylori regulating autophagy and apoptosis of colon cancer epithelial cells through NF-κB signaling pathway. Helicobacter pylori (H. pylori, Hp) and human colon cancer epithelial CACO-2 cell lines were cultured in vitro. Hp treatment 0 h, 3 h, 6 h, 9 h and control group, 18 μmol·L-1NF-κB specific inhibitors were collected The CACO-2 cells treated with SN50 for 6 h and 18 μmol·L-1SN50 + Hp for 6 h were used to detect the autophagy rate and apoptosis rate of each group of cells by flow cytometry; the CACO-2 cells after Hp gradient time treatment were collected to extract each The total protein was detected by Western blot (Western Blot) to detect the expression of autophagy-related proteins Beclin1related X protein (Bax) and Nrf2. The autophagy rate and apoptosis rate of CACO-2 cells gradually increased with the extension of Hp action time, and there was a statistically significant difference between each time point and 0 h (P <0.01). The expressions of autophagy-related proteins Beclin1, apoptosis-related proteins Bax and Nrf2 gradually increased with the extension of Hp action time. Compared with 0 h, the difference in protein expression at each detection time point was statistically significant (P<0.01). Compared with the control group, the autophagy rate and apoptosis rate of CACO-2 cells were significantly enhanced after Hp treatment; the autophagy rate and apoptosis rate% of CACO-2 cells were significantly reduced after treatment with Sn50; after the combined treatment with Hp and Sn50, the autophagy of CACO-2 cells The rate and apoptosis rate were significantly higher than the control group, but lower than the Hp alone treatment group, the difference was statistically significant. Hp stimulation significantly promotes autophagy and apoptosis of CACO-2 cells. The mechanism may be related to the activation of NF-κB pathway.

Keywords: Helicobacter pylori; epithelial cells; autophagy; apoptosis; Nrf2

Introduction

The main factor leading to colon cancer is helicobacter pylori (Helicobacter pyroli, Hp) infection. It is also closely related to colon cancer, which is associated with colon cancer. Helicobacter pylori has grown into a carcinogenic factor of class I [1-2]. H rate. H rate. In my country, pylori infection is approximately 53%, and is on the rise [3]. H. The infection with pylori has seriously affected human health. No efficient method for completely eliminating H has currently been found. Pylori infection, while the pathogens of Hp are still unclear, is important in finding an effective therapeutic treatment for Hp infection by examining the mechanism of Hp in depth and distributing the pathogensic mechanism.

Autophagy is a body self-protection defence mechanism. It is involved in the regulation of the body 's growth, distinction, development of various diseases and occurrence. The resistance to pathogens is important[4-5]. It plays an important role. Apoptosis is a genetically controlled process of programmed cell death. Research has found that in Positive Hp infection, apoptosis of colon cancer cells is significantly higher than in negative infection patients[6]. The triggering switch for host cell inflammation may include autophagy and apoptosis

[a] School of Medicine, University of Electronic Science and Technology；Department of Gastrointestinal Surgery, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital，chengdu, Sichuan,610072, China
[b] Medicine, University of Electronic Science and Technology；Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital，chengdu, Sichuan,610072, China
Corresponding author: ShaoPing Deng
Address: School of Medicine, University of Electronic Science and Technology of China, Sichuan Academy of Medical Sciences·Sichuan Provincial People's Hospital/610072,China
Email: 954930600@qq.com

caused by bacterial infection, but the autopathy mechanism and apoptosis of colon-epithelial cells caused by Hp infection are not clear. The relationship of autophagy HP infections with CACO-2 Apoptose of CACO-2 cells was investigated in this study, and the possible mechanisms of action were studied with a specific Nrf2 inhibitor, SN50, to provide the clinical prevention and treatment reference for PH infections. In this study, HP was infected with CACO-2.

Materials and Methods

Materials

American ATCC purchased HP strains; Shanghai Institute of Cellular sciences (SACC2) purchased CACO-2 human cell line; Hylon of the United States was purchased from the Hyllon medium and Trypsin; Vancomycin (K0059), Polymyxin (P104), Acride orange (BNN2017) were purchased from Sigma in the United States. Sorcer; UVP analysis system GDS7500 has been purchased from UVP. Sorcer; UVP system.

Method

Hp cultivation

Remove the frozen Hp strain from the liquid nitrogen tank, and quickly place it in a 37 ℃ water bath to gently shake it to fully melt. After centrifugation at 3,000 r·min for 5 min, draw 50 μL and discard the supernatant. Mix by pipetting and inoculate HP into brain heart infusion blood agar plate, place at 37 ℃, microaerobic (10% carbon dioxide, 5% oxygen, 85% nitrogen) cultured for 48 hours under culture conditions. Pick colonies into 20 mL of sterile Hp liquid medium and place on a shaker in a three-gas incubator under micro-aerobic conditions, 37 °C, 100 r·min for 24 to 30 h.

CACO-2 culture of colon cancer epithelial cells

Remove frozen cacocytes and mix with F12 medium, centrifuge the pellet to collect the cell pellets, use the fresh cell culture Resuspense the pellet, add the re-substitute solution to the cell culture medium F12 containing 10% bovine foetal serum, and place it in a 37 ° C, 5% serum frozzy, CACO-2 liquids, quickly place into 37 ° C water and gently shake to melt. If the cells reach 80% of the bottom of the dish, discard the old culture solution, remove phosphate buffered Saline (PBS) and digest with 0.25% trypsin. Suspend the solution of cell culture at 1:4 Subculture.

Method of HP infection of CACO-2 cells

Take CACO-2 cells in the logarithmic growth phase, digest with 0.25% trypsin and centrifuge to collect the cells. After resuspending the cell culture solution, adjust the cell concentration to 2 × 105 cells/ml under the microscope and inoculate 2 mL per well in 6-well plates. , Placed in 37 ℃, 5% carbon dioxide incubator. Liquid culture Hp 24 h, UV spectrophotometer to detect bacterial concentration (1A 600 = 1 × 108CFU·mL-1). When the CACO-2 cells grow to 80% of the bottom of the dish, replace the fresh medium, and add the H. pylori suspension according to the number of bacteria: number of cells = 100:1, place them under 37 ℃, 5% carbon dioxide, co-culture for 0 h, 3h, 6 h, 9 h.

Flow cytometry detection of autophagy in CACO-2 cells

The cell was added with 1 mg·ml-Acridine orange solution 1, after 15 min incubation at 37 ° C in the dark, the cell washed up at 1 to 2 times with PBs, 100 mg μl of pre-cooled PBS sol, after 15 minutes of incubation, with 0.25% of trypsin, after 37 ° C of dark cell in the black cell.

Flow cytometry detection of CACO-2 apoptosis

The HGS time-gradient-treated CACO-2 cells, control CACO-2 cells, 18 μmol·L-1SN50 treated 6 h CACO-2 cells, 18 μmol·L-1SN50 + Hp treated total 6 h CACO-2 cells, 0.25% pancreatin digestion After adjusting the cell concentration to 2 × 106 cells/ml under the microscope, take 1 mL of cell suspension and centrifuge and collect the pellet. After washing twice with PBS, add 500 μL of binding buffer to resuspend the cells and add 10 μL of phospholipid-binding protein Anexin V-FITC Mix with 5 μL of propidium iodide (PI), and incubate for 15 min at room temperature in the dark. Flow cytometry was used to detect apoptosis.

Detection of protein expression by Western blot

CACO-2 cells were digested by 0.25% trypsin, and pellets were collected. Hp time gradient was used. The cells were put on ice for 30 minutes, 4 ° C, 12 000r·min-1, after adding the cell lysate and centrifuging them for 20 minutes to take the supernatant and the BCA. The method of detecting the concentration of protein in the surnatant. Take 50 μg of protein in a volume ratio of 4:1, add 5 μg per buffer loading, boil at 100 ° C for 5 min to make protein specimen. The protein was electrophored vertically after the sample was loaded. The electrophoretic process was continued with 120 V until the glass plate was removed by bromophhenol blue after electrophoresis was at 80 V during the 30 minutes. Remove the protein gel, transfers to 100 V ice bath, incubate 5 percent skim milk powder for 6

hours at room temperature, add the appropriate primary antibody (1:500) and shake for 4 ° Cover night, wash with TBST solution washing and add horseradish peroxide The secondary enzyme antibody (1:1 000) was incubated for 1 hour at 37 ° C. ECL luminescent agent was added for development after the washing solution using TBSST. Images were gathered via an automatic system of gel imaging.

Statistical method

The data were analysed by statistical software SPSS 21.0. The measurement data were expressed as x ±s in accordance with normal distribution. A variance analysis was carried out with a single-factor comparison between multiple groups, and the LSD-t test was applied in pairs. Statistically significant was considered $P < 0.05$.

Results

The effect of Hp infection on autophagy of CACO-2 cells

Hp with oranges stimulates cells of colon r epithelial CACO-2 at 0 h, three hours, six hours and nine hours. Figure 1 shows statistical results. The difference was statistically significant over all four periods. After Hp stimulation, with an extension of Hp action time, the autophagy rate of CCO-2 cells has gradually increased. Statistical differences were observed between 3 h, 6 h, 9 h and 0 h for Hp stimulation.

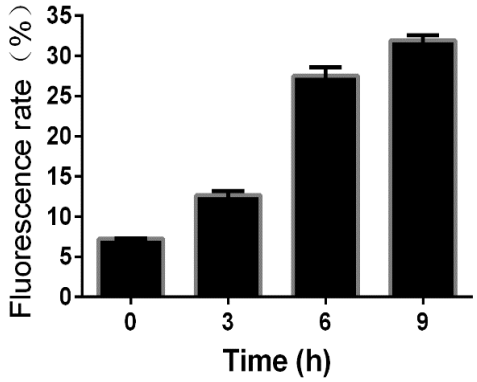

Figure 1. **Flow cytometry detection of autophagy rate of CACO-2 cells.**

Effect of Hp infection on apoptosis of CACO-2 cells

The CACO-2 cells stimulated by Hp for 0, 3, 6, and 9 h were collected, and flow cytometry was used to detect the apoptosis rate of CACO-2 cells at various time points. The results are shown in Figure 2. The overall difference between the four time periods is statistically significant. After Hp stimulation, the apoptosis rate of CACO-2 cells gradually increases with the extension of Hp action time. The difference in Hp stimulation at 3 h, 6 h, 9 h and 0 h is statistically significant. significance.

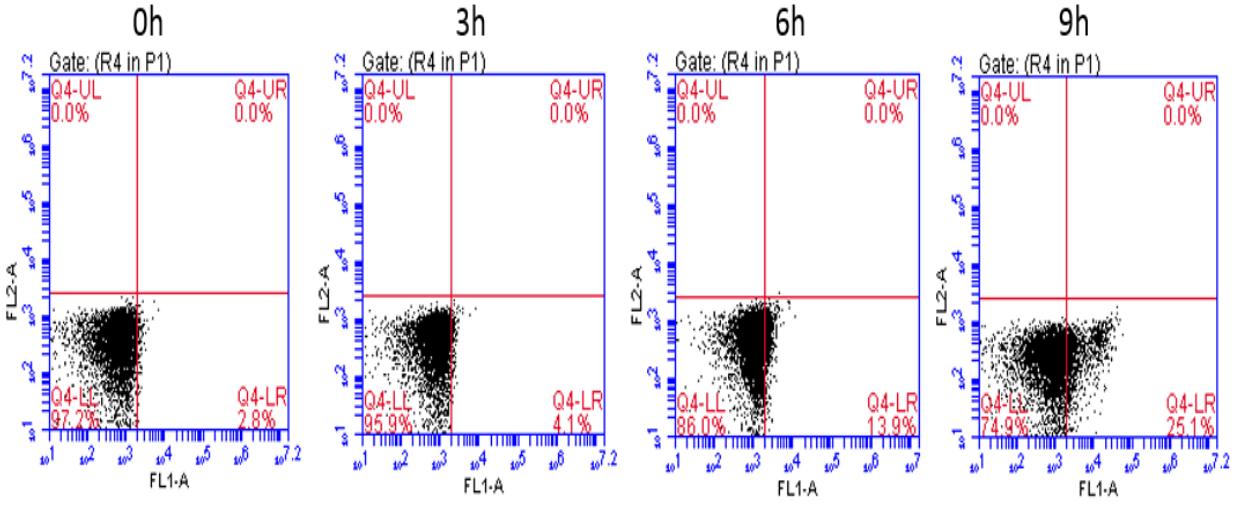

Figure 2. **Flow cytometry was used to detect the apoptosis rate of CACO-2 cells.**

Effect of Hp infection on the expression of Nrf2 pathway protein Hp

Each cell group was collected for the extraction of the total protein of each group after action on cells CACO-2 for 0 h, 3 h, 6 h and 9 h. The four times of 0-h, 3-h, 6-h, and 9-hour protein expression of Nrf2, Beclin1, Bax is the following. Figure 3 shows the results. In Nrf2, Beclin1, Bax protein expression, there were statistically significant differences between 0 h, 3 h, 6 h and 9 h

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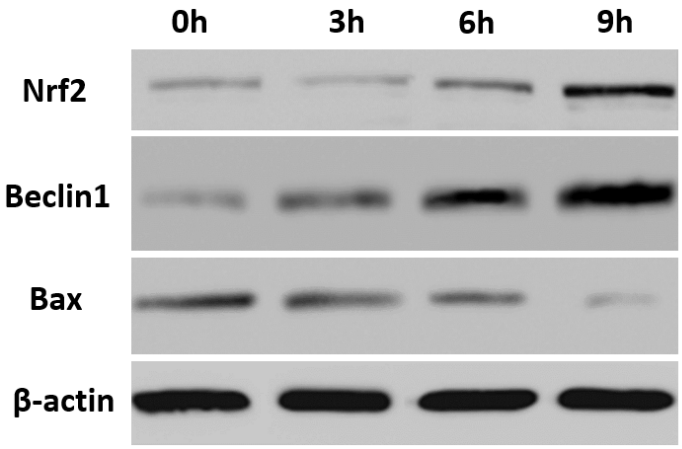

Figure 3. **Western blot method to detect the expression of Nrf2 pathway protein.**

Hp infection combined with Nrf2 inhibitor on autophagy of CACO-2 cells

The results of the Nrf2 pathway inhibitor SN50 and Hp for co- treatment of CACO-2 cells for 6 hours were found to detect Hp and apoptosis of each group of cells and the results are shown in Figure 4. Facilitation of CACO-2 cell autophagy and apopticosy of each cell group is detected in Figure 4. The autophagy and apoptosis in the four groups of cells in CACO-2 was statistically significant. CACO-2 cells were significantly more autophagized after treatment with Hp compared with autophagy and apoptosis, CACO-2 cells were substantial weakens in autophagy and apoptosis following SN50 treatment; CACO-2 cells, autophagy following the treatment of Hp and SN50 in combination with the control group was significant in comparison with that of the CACO-2 control group. The difference has been significant statistically.

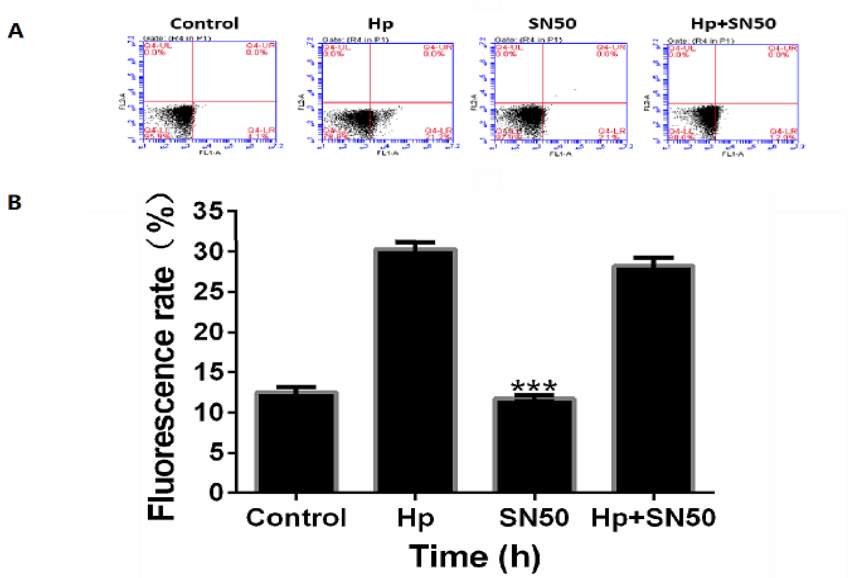

Figure 5. **Effect of Hp and SN50 treatment on cell autophagy and apoptosis: A. Flow cytometry to detect cell apoptosis; B. Statistics of cell autophagy rate.**

Discussion

Autophagy is a ubiquitous phenomenon in eukaryotic cells, which depends on the self-digestion and recycling mechanism of lysosomes and is highly conservative during evolution [7]. The occurrence of autophagy begins with the formation of autophagosomes, which are formed by the double membranes of cells enclosing the degradation products, which are digested and degraded after fusion with lysosomes [8]. Autophagy can balance the synthesis and metabolism of cells and play a role in stabilizing the

intracellular environment. Autophagy also participates in regulating the innate immunity of cells and the process of acquired immune response [9-10].

After Hp infects colon cancer epithelial cells, the cells will induce the production of autophagy in order to eliminate the pathogenic bacteria, to degrade the pathogens and the toxic substances they secrete, and protect the gastric epithelial cells [11]. Acridine orange can enter lysosomes and autophagosomes and can be protonated. Cells labeled with acridine orange appear green when no autophagolysosomes are produced, otherwise they appear red. Flow cytometry is used for quantitative detection .The rate of positive fluorescent cells labeled with acridine orange is the autophagy rate of the cells [12]. In this study, acridine orange labeled gastric epithelial CACO-2 cells infected with Hp for 0 h, 3 h, 6 h, and 9 h, and flow cytometry was used to detect the rate of autophagy. The results showed that after Hp stimulation for 3 h, CACO-2 cells showed autophagy. Phenomenon, the cell autophagy rate gradually increased with the extension of Hp action time, the cell autophagy rate reached a peak at 6 h, and then began to decline, indicating that cells can quickly initiate the occurrence of autophagy early in Hp infection, in order to promote the pathogenic bacteria Clear. Beclin 1 protein participates in the formation of autophagy precursors, guides the positioning of autophagy-related proteins on the autophagosome membrane, and thus promotes the progress of autophagy. It is an important factor regulating the occurrence of autophagy, and its expression reflects to a certain extent[13-14]. The results of this study showed that the expressions of phagocytic-related proteins Beclin1 gradually increased with the extension of Hp stimulation time. Hp treatment for 6 h had the most significant effect on protein expression. It indicated that Hp infection of CACO-2 cells promoted the occurrence of autophagy.

Conclusions

H. pylori infection promotes the occurrence of autophagy and apoptosis of colon cancer cells CACO-2, and its mechanism of action is related to the activation of the Nrf2 pathway. The above results may be clinically developed for new prevention and treatment Drugs infected with HP provide a theoretical basis.

References

[1] Antonio, Ponzetto, Natale, et al. Screening for Gastric Cancer. [J]. American Journal of Gastroenterology, 2019.

[2] Fritsche J, Weinschenk T, Walter S, et al. Immunotherapy against several tumors including gastrointestinal and gastric cancer: CA 2018.

[3] Levine B, Kroemer G. Autophagy in the pathogenesis of disease. [J]. Cell, 2008, 132(1):27-42.

[4] Liang X H, Jackson S, Seaman M, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. [J]. Nature, 1999, 402(6762):672.

[5] White E, Dipaola R S. The Double-Edged Sword of Autophagy Modulation in Cancer[J]. Clinical Cancer Research, 2009, 15(17):5308-5316.

[6] Bilgic I, Ozalp N, Tez M, et al. Serum bFGF concentrations in gastric cancer patients[J]. Bratislavské Lekárske Listy, 2008, 109(1):8.

[7] M, Chiara, Maiuri, et al. Self-eating and self-killing: crosstalk between autophagy and apoptosis. [J]. Nature Reviews Molecular Cell Biology, 2007.

[8] Bhat P, Kriel J, Shubha Priya B, et al. Modulating autophagy in cancer therapy: Advancements and challenges for cancer cell death sensitization[J]. Biochemical Pharmacology, 2018.

[9] Bilgic I, Ozalp N, Tez M, et al. Serum bFGF concentrations in gastric cancer patients[J]. Bratislavské Lekárske Listy, 2008, 109(1):8.

[10] Shao G Q, Zhang N Z. Effect of somatostatin on expression of VEGF and b FGF in human gastric cancer xenografts in nude mice[J]. World Chinese Journal of Digestology, 2014, 22(36):5659-5664.

[11] De Paulis A, Prevete N, Rossi F W, et al. Helicobacter pylori Hp (2–20) Promotes Migration and Proliferation of Gastric Epithelial Cells by Interacting with Formyl Peptide Receptors In Vitro and Accelerates Gastric Mucosal Healing In Vivo[J]. Journal of Immunology, 2009, 183(6): 3761-3769.

[12] Hayashi M, Sofuni T, Ishidate M. An application of Acridine Orange fluorescent staining to the micronucleus test. [J]. Mutation Research Letters, 1983, 120(4):241-247.

[13] Guo G F, Jiang W Q, Zhang B, et al. Autophagy-related proteins Beclin-1 and LC3 predict cetuximab efficacy in advanced colorectal cancer[J]. World Journal of Gastroenterology, 2011, 17(43):4779.

[14] Liu K, Shi Y, Guo X, et al. CHOP mediates ASPP2-induced autophagic apoptosis in hepatoma cells by releasing Beclin-1 from Bcl-2 and inducing nuclear translocation of Bcl-2[J]. Cell Death & Disease, 2014, 5(7): e1323.