Microbial Pathogenesis 95 (2016) 77–81

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Functional profile of gastric epithelial cells infected with *Helicobacter pylori* strains

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ARTICLE INFO

Article history:
Received 19 January 2016
Received in revised form
21 March 2016
Accepted 22 March 2016
Available online 23 March 2016

Keywords:
Helicobacter pylori
Apoptosis
Proliferation
Adhesion
Migration

ABSTRACT

Helicobacter pylori infection represents a key factor in the etiology of various gastro-duodenal diseases, ranging from chronic gastritis to the development of peptic ulcer disease and end-stage gastric cancer. In the present study, the 26695 and SS1 strains of *H. pylori* were used to study the differential functional profiles of gastric epithelial cells infected with *H. pylori*. The apoptosis rates in GES-1 cells were significantly increased 3, 12 and 24 h after *H. pylori* 26695 and SS1 infection. Moreover, apoptosis by cells infected with the *H. pylori* 26695 strain was significantly higher than cells infected with the SS1 strain of *H. pylori*. No significant changes in the proliferation rates of GES-1 cells were observed after *H. pylori* 26695 or SS1 infection at any time during the experimental period. Exposure to *H. pylori* 26695 and SS1 induced a significant decline in the adhesion rates of GES-1 cells in a time-dependent manner. Furthermore, *H. pylori* 26695 infection increased migration of GES-1 cells every hour during the whole experimental period compared with control cells. However, GES-1 cells infected with the *H. pylori* SS1 strain exhibited migration rates almost stable and comparable to those of control cells. These results indicate that the gastric epithelial cells respond differently depending on the *H. pylori* strains. This study indicates that the development of different gastric-related diseases may be a *H. pylori* strain-specific response.

1. Introduction

Helicobacter pylori is one of the most prevalent pathogens that contribute to human diseases [1,2]. According to epidemiological studies, approximately 50%–80% of the world population is infected with *H. pylori*, and areas of high incidence are concentrated in East Asia, including China, Japan and South Korea [3,4]. Chronic infection with *H. pylori* can result in DNA damage and genetic instability of the gastric mucosal cells, causing chronic gastritis and atrophy. The International Agency for Cancer Research (IACR) classified *H. pylori* as a class I carcinogen in 1994 [5], as numerous studies evidenced the relationship of *H. pylori* and gastric cancer from a combination of environmental and host-dependent factors.

The infection strategy of *H. pylori* and the defense capacity of the gastric epithelial cells in the gastric mucosa collectively influence colonization, survival, and development of *H. pylori* infection-derived diseases. Mucosal epithelial cells are not only responsible for digestive processes, but also exert the crucial function of protecting the underlying tissue from pathogenic microorganisms that otherwise would invade the lumen [6]. Gastric epithelial cells form the first line of defense, thus contributing an innate defense such as cell barrier integrity, cell turnover, autophagy, and innate immune responses [6]. However, *H. pylori* can break through the gastric mucosal barrier and remain as a persistent infection via highly specialized mechanisms, which facilitate *H. pylori* to avoid host defense mechanisms [7]. The failure of the host response in clearing the *H. pylori* infection promotes the development of chronic infection such as chronic gastritis.

H. pylori infection represents a key factor in the etiology of various gastro-duodenal diseases, ranging from chronic gastritis to the development of peptic ulcer disease and gastric cancer (GC) [8]. The significant variation of the different strains of *H. pylori* might be one of the important inducing factors that determines prevalence and incidence of *H. pylori*-associated diseases [9,10]. Determining the effects of the different *H. pylori* strains on the biological function of gastric epithelial cells will allow for better understanding of their cytotoxic effect and their regulatory mechanisms including the integration and rehabilitation of the mucosal epithelial barrier.

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http://dx.doi.org/10.1016/j.micpath.2016.03.007
0882-4010/

In this context, the present study was performed with the following objectives (1) to investigate the time-response in cell apoptosis and proliferation in gastric epithelial cells (GES-1) after *H. pylori* infection, (2) to characterize cell adhesion and migration rates in GES-1 cells after *H. pylori* infection, (3) to further explore the cytotoxic diversity of *H. pylori* strains.

2. Materials and methods

2.1. H. pylori

Human-adapted strains of *H. pylori* 26695 and SS1, were provided by the *H. pylori* Research Laboratory of the Chinese Center for Disease Control and Prevention (Beijing, China). *H. pylori* 26695 strain was originally isolated from a patient in the United Kingdom with gastritis and Jcan-F Tomb et al. obtained the complete genome sequence of the gastric pathogen *H. pylori* 26695 in 1997 [11]. *H. Pylori* SS1 came from a 42-year-old Greek-born woman in 1997, who had been diagnosed with a peptic ulcer [12]. Introduced *H. Pylori* were grown on chocolate agar plates supplemented with 10% sheep's blood at 37 °C under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) and subcultured every 3 days. For infection, *H. pylori* was harvested in chocolate agar broth and quantified by a spectrophotometer reaching 1×10^8 CFU/ml.

2.2. Human gastric epithelial cells

The human gastric epithelial cell line GES-1 was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and maintained in Dulbecco Modified Essential Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) under a humidified atmosphere of 5% CO_2 at 37 °C. When cells reached approximately 80% confluence in 25 cm^2 culture flasks, cells were treated with 0.25% trypsin and passaged.

2.3. Co-culture of GES-1 cells with H. pylori

GES-1 cells were plated onto 6-well plates in DMEM containing 10% FBS (1×10^5 cells/2ml/well) overnight. The 26695 and SS1 strains of *H. pylori* were harvested and suspended in DMEM (including 10% FBS but no antimicrobial agents). Bacteria were added into the cells at a multiplicity of infection (MOI) of 200:1 and co-cultured for 0, 3, 12 and 24 h. GES-1 cells were immediately used to detect cell apoptosis, proliferation, adhesion and motility rates [13].

2.4. Cell apoptosis assays

GES-1 cell apoptosis assay was performed using the Annexin V-FITC Apoptosis Detection Kit (KeyGEN BioTECH, Nanjing, China) according to the instructions provided by the manufacturer. Briefly, 1×10^5 GES-1 cells were mixed together with 500 μl of Annexin binding buffer, 5 μl of FITC-conjugated Annexin V antibody and 5 μl of propidium iodide (PI). The mixture was incubated in the dark for 15 min at room temperature. The relative number of apoptotic cells was determined using flow cytometry [14].

2.5. EdU cell proliferation assay

Cell proliferation was measured using a key fluor 488-EdU Proliferation Detection Kit (KeyGEN BioTECH, Nanjing, China) according to the manufacturer's instructions. GES-1 cells were incubated with *H. pylori* at 200:1 of bacterium to cell ratio in DMEM containing 10% FBS for 0, 3, 12 and 24 h. All cells were treated with 50 μmol/L of EdU for 3 h at 37 °C. After being fixed, the treated cells on coverslips were permeabilized with 0.5% TritonX-100 for 10 min. Cells were stained with Click-iT reaction mixture and incubated with Hoechst 33342 to stain the cell nuclei. Images were captured using inverted fluorescence microscope (Olympus, Tokyo, Japan) [15]. The numbers of EdU- and DAPI-positive cells were quantified by IMAGEJ software. The percentages of immunopositive cells [(the number of immunopositive cells/total cells) × 100] were expressed. Five random fields of each well were chosen to perform the calculation.

2.6. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for cell adhesion

A 96-well plate was treated with matrigel (0.04 mg/mL, 50 μl) (BD Biosciences, San Jose, CA) in DMEM overnight to facilitate cell attachment. GES-1 cells were cultured with the 26695 and SS1 strains of *H. pylori* for 0, 3, 12 and 24 h, and then trypsinized and seeded into 100 mL of culture medium. MTT solution (5 mg/mL, 20 μL) was added to the cells, and plates were further incubated at 37 °C for 4 h. The supernatant was carefully removed, and dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO) was added to each well to dissolve formazan crystals. After 10 min shaking, the optical density was measured using ELISA at 490 nm wavelength. Cell adhesion rates = (OD of the infection group cells/OD of the control group cells) x 100% [16].

2.7. Scratch motility (wound-healing) assay

GES-1 cells (1×10^5 cells/well) were cultured on glass bottom dishes and allowed to form a confluent monolayer for 24 h. Cells were then scratched using a sterile 10 μL pipette tip and the edge of each gap was straight and smooth. Single Cell Transferred Speed (SCTS) of treated cells were observed under the Living Cell Working Station. Six random fields were selected and imaged continuously taken every 10 min for a period of 24 h. Afterwards, the migrated distances were measured and the single cell migration velocity was calculated by manual counting. The analysis of image data was obtained by the Image® Pro Plus 6.2.

2.8. Statistical analysis

The SPSS version 16.0 was used for statistical analysis. Continuous variables were expressed as mean ± standard deviation (SD) and the differences between the two data sets were determined by the Student's *t*-test. A $P < 0.05$ (two-tailed) was considered statistically significant.

3. Results

3.1. Apoptosis in H. pylori-infected GES-1 cells

The apoptosis rates in GES-1 cells were significantly increased 3, 12 and 24 h after infection with the 26695 and SS1 strains of *H. pylori* (Fig. 1A and Fig. 1B). A cell apoptosis rate of 31.83 ± 4.39% was observed in GES-1 cells 3 h after *H. pylori* infection with the 26695 strain, whereas the apoptosis rate was 5.00 ± 3.15% in control GES cells. At the 12 and 24 h time-point after *H. pylori* 26695 infection, cell apoptosis increased to 50.80 ± 7.54% and 54.00 ± 6.41%, respectively, in other words, a 10-fold ($P < 0.01$) and a 11-fold ($P < 0.01$) increase compared with the control group. GES-1 cells infected with the strain SS1 of *H. pylori* displayed apoptotic rates of 12.80 ± 1.85%, 15.23 ± 3.18% and 33.30 ± 5.89% at 3, 12, and 24 h after infection, respectively. This represented a significant increase ($P < 0.01$) compared with the control group. Altogether, the cell apoptosis rates in the 26695 strain of *H. pylori*-treated GES cells were significantly higher than the ones treated with the SS1 strain ($P < 0.05$).