Apoptosis, proliferation and \( p53 \) gene expression of \( H.\) pylori associated gastric epithelial lesions

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Abstract

AIM: To study the relationship between Helicobacter pylori (\( H.\) pylori) and gastric carcinoma and its possible pathogenesis by \( H.\) pylori.

METHODS: DNEL technique and immunohistochemical technique were used to study the state of apoptosis, proliferation and \( p53 \) gene expression. A total of 100 gastric mucosal biopsy specimens, including 20 normal mucosa, 30 \( H.\) pylori-negative and 30 \( H.\) pylori-positive gastric precancerous lesions along with 20 gastric carcinomas were studied.

RESULTS: There were several apoptotic cells in the superficialic pithelium and a few proliferative cells within the neck of gastric glands, and in \( \alpha \) \( p53 \) protein expression in normal mucosa. In gastric carcinoma, there were few apoptotic cells, while there was a large number of proliferative cells, and expression of \( p53 \) protein significantly was increased. In the phase of metaplasia, the apoptotic index (AI, 4.36\%±1.95\%), proliferative index (PI, 19.11\%±6.79\%) and positivity of \( p53 \) expression (46.7\%) in \( H.\) pylori-positive group were higher than those in normal mucosa (\( P<0.01 \)).

AI in \( H.\) pylori-positive group was higher than that in \( H.\) pylori-negative group (3.81\%±1.76\%), PI in \( H.\) pylori-positive group was higher than that in \( H.\) pylori-negative group (12.25\%±5.63\%, \( P<0.01 \)).

In the phase of dysplasia, AI (2.31\%±1.10\%) in \( H.\) pylori-positive group was lower (3.05\%±1.29\%) than that in \( H.\) pylori-negative group, but PI (33.89\%±11.6\%) was significantly higher (22.09±8018\%, \( P<0.01 \)).

In phases of metaplasia, dysplasia and gastric cancer in the \( H.\) pylori-positive group, AIs had an evidently gradual all decreasing trend (\( P<0.01 \)), while PIs had an evidently gradual increasing trend (\( P<0.05 \) or \( P<0.01 \)), and there was also a trend of gradual increase in the expression of \( p53 \) gene.

CONCLUSION: In the course of the formation of gastric carcinoma, proliferation of gastric mucosa can be greatly increased by \( H.\) pylori, and \( H.\) pylori can induce apoptosis in the phase of metaplasia, but in the phase of dysplasia \( H.\) pylori can inhibit cellular apoptosis. And \( H.\) pylori infection can strengthen the expression of mutated \( p53 \) gene.

Subject headings Helicobacter pylori; gastric precancerous lesion; apoptosis; proliferation; \( p53 \) gene


INTRODUCTION

\( H.\) pylori infection is epidemiologically associated with the development of gastric cancer\(^1\), but it is unknown how \( H.\) pylori does so. In this study, DNEL technique\(^8\) and immunohistochemical staining were used to dynamically observe and compare the state of apoptosis, proliferation and \( p53 \) gene expression in \( H.\) pylori-negative or \( H.\) pylori-positive gastric precancerous lesion as well as gastric carcinomas. The purpose is to probe into the effect of \( H.\) pylori on apoptosis, proliferation and \( p53 \) gene expression in gastric epithelium and to find out the relationship between \( H.\) pylori and gastric carcinogenesis, and the possible mechanism.

MATERIALS AND METHODS

Subjects

All samples were selected from people screened by endoscopy in a high risk area of gastric carcinoma in Zhuanghe, Liaoning Province. A total of 100 gastric mucosal biopsy specimens, including 20 normal mucosa, 30 metaplasia, 30 dysplasia and 20 gastric carcinoma cases.

\( H.\) pylori infecti on was assessed by hematoxylin-eosin staining\(^9\) and PCR\(^11\). If both results of the tests in a patient were positive, the patient was considered to be infected by \( H.\) pylori; if neither was positive, the patient was considered negative.

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end la belling method

Using the kit (Oncor, San Diego, USA), the staining steps are as follows: ① the sections were deparaffinised through xylene and alcohol, and washed; ② diges ted for 15 min with proteinase K (20 mg·L\(^{-1}\)); ③ quenched with 30 ML·L\(^{-1}\) H\(_2\)O\(_2\); for 20 min; ④ applied with equilibration buffer for 15 s at room temperature; ⑤ added with terminal deoxynucleotidyl transferase (TdT) and incubated at 37\(^\circ\)C for 60 min; ⑥ added with STOP/WASP buffer at 37\(^\circ\)C for 10 min; ⑦ dropped with hydrogen peroxidase for 30 min at room temperature; and ⑧ visualized by immersi on in 3,3’,-diaminobenzidine (DAB) solution, restained with methyl-green, and dehyd rated, transparency, mounted. PBS was substituted for TdT as negative control.

Immunohistochemical staining

SP kit was used (Zymed, USA). The primary a nitibodies were PCNA monoclonal antibody (diluted 1:50) and \( p53 \) monoclonal anti body (ready to use, Maixin, Fujian), respectively. Before staining, the sections were microwave heated in 0.05 mol·L\(^{-1}\) citric acid solution for antigen retrieval. PBS was substituted for primary antibodies as negative control.
**Observed parameters**

Two samples were stained by DNEL and immunohistochemical staining, then the DNEL-positive cells (apoptotic cell) and PCNA-positive cells (proliferative cell) were observed. Apoptotic index (AI) and proliferative index (PI) were obtained by calculating the percentage of positively stained cells evaluated for each tissue section after counting 1000 cells at more than 5 high power fields.

**Statistical analysis**

A t test was used to compare the means. The positivity of p53 protein was analyzed by X² test.

**RESULTS**

**Characteristics of DNEL-positive, PCNA-positive and p53 protein-positive cells under microscopy**

DNEL-positive cells (apoptotic cell) appeared brown corpuscular or diffuse in cell nuclei, and individual thickening nuclear membrane appeared brown. PCNA-positive cells (proliferative cell) appeared brown corpuscular in cell nuclei. Positive product of p53 expression was restricted in cell nuclei. In normal mucosa, apoptotic cells sporadically scattered on the epithelium, a few proliferative cells scattered on the glandular neck and expression of p53 protein was not seen. But in the tissue of gastric carcinoma, apoptotic cells accounted for 1.62%; proliferative cells for 41.99% and a cluster was formed all over the lesions; there was a significant increase in the expression of p53 protein. Effect of H. pylori infection on apoptosis in gastric epithelium. In the metaplasia mucosa, the apoptotic index in H. pylori-positive group was higher than that in normal mucosa (P<0.01), and it was also higher than that in H. pylori-negative group; while in the dysplasia mucosa, AI in H. pylori-positive group was lower than that in H. pylori-negative group. In the metaplasia, dysplasia mucosa and gastric carcinoma AI presented with an evidently gradual decrease trend (P<0.05 or P<0.01 Table 1) in H. pylori-positive group.

**Effect of H.pylori infection on proliferation in gastric epithelium**

In metaplasia and dysplasia mucosa the PI was significant higher than those in normal mucosa (P<0.01), and that in H. pylori-positive group was higher than that in H. pylori-negative group (P<0.01). From the normal mucosa to the gastric carcinoma, the PI has a gradual increase trend (P<0.05 or P<0.01, Table 2) in H. pylori-positive group.

**Effect of H.pylori infection on the expression of p53 in gastric epithelium**

In the metaplasia mucosa, the positivity of p53 protein expression in H. pylori-positive group was higher than that in normal mucosa (P<0.01). In the metaplasia, dysplasia mucosa and gastric carcinoma, there was a trend of gradual increase in positivity of p53 protein expression (Table 3) in H. pylori-positive group.

**DISCUSSION**

Gastric mucosa consists of continuously renewed cells and cell proliferation and apoptosis maintain their balance[14]. This study shows that the apoptotic cells were identified in gastric surface epithelium and formed “an apoptotic zone”; proliferative cells were seen in the neck region of the mucosal glands and formed “a proliferation zone”. This distribution shows the proliferating zone gradually maturing, aging and death to the surface in the gastric mucosal epithelium. However in gastric carcinoma, apoptotic cells amount to 1.62%, proliferative cells amount to 41.99%, which clustered all over the tumor tissue. This change obviously lost the distribution characteristics of apoptotic zone and proliferating zone which elucidates that the regulation of apoptosis and proliferation have already been beyond the normal mucosa and appear significantly disordered in gastric carcinoma.

Human gastric carcinogenesis is a multistep and multifactorial process[16-17]. In this process, the state of apoptosis and proliferation of gastric epithelium will change[18,19]. In this study, H. pylori infection was found to affect the cell apoptosis and proliferation. In the metaplasia mucosa, AI was higher than that in normal mucosa in H. pylori-positive group, and higher than that in H. pylori-negative one; however, in the dysplasia mucosa, AI in H. pylori-positive group is lower than that in H. pylori-negative group. In the process of gastric carcinogenesis[18,19], from the phase of metaplasia, dysplasia to gastric carcinoma, AI gradually decreased in H. pylori-positive group. This shows that from normal mucosa to gastric carcinoma, H. pylori may induce cell apoptosis in the phase of metaplasia; but it inhibits cell apoptosis in the phase of dysplasia, this is familiar with gastric carcinoma. Several reports suggest that H. pylori produces cytoxic protein (CagA and VacA)[20-24], and gastric mucosa can increase some cytokines, nitrous oxide synthetase and oxygen radicals released after H. pylori infection[25-26]. At the same time, H. pylori infection can lower the gastric antioxidant ability. All those factors make DNA dam age or enhance the susceptibility of DNA-damage, and those DNA-damaged cells can be...
cleared away by apoptosis\textsuperscript{[16]} and this may be the mechanism that \textit{H. pylori} in duces apoptosis. It has been proved that wild type p53 protein can induce cell apoptosis but the intracellular accumulation of mutant p53 protein can inhibit cell apoptosis and promote cell transformation and proliferation, resulting in carcinogenesis\textsuperscript{[8]-[11]}. In this study, in the phase of metaplasia, positivity of p53 protein expression in \textit{H. pylori}-positive group was higher than that in normal mucosa. From the phase of metaplasia to gastric carcinoma, the infection and gastric carcinoma in order to decrease the incidence rate of \textit{H. pylori} infection may be an important factor of gastric carcinogenesis. This makes it clear that \textit{H. pylori} infection can strengthen the expression of p53 gene. With the accumulation of mutant and expression of p53, its inhibiting effect on apoptosis\textsuperscript{[12]-[14]} will overpass the induction effect of \textit{H. pylori}. Therefore in the \textit{H. pylori}-positive dysplasia, there was a decrease in apoptosis. In this study, in the \textit{H. pylori}-positive gastric precancerous lesions-metaplasia and dysplasia, PI was significantly higher than that in normal mucosa, and higher than those in the c orresponding negative lesions. In the progress from normal mucosa to the malignant phenotype, PI gradually increased in \textit{H. pylori}-positive group. T herefore suggests that \textit{H. pylori} can induce apoptosis and proliferation in the gastric epithelial cells, which increase the instability of gastric mucosa and carcinoma variability. Accompanied with the progress of lesions and the accumulation of p53 protein, \textit{H. pylori} induces gastric epithelial cell hyperproliferation and apoptosis reduction or even imbalance of the apoptotic and proliferative process, and accumulates at ion of DNA-damaged cells, ultimately resulting in gastric carcinogenesis. This makes it clear that \textit{H. pylori} infection may be an important factor of gastric cancer. Thus, it is significant to prevent \textit{H. pylori} infection, eradicate \textit{H. pylori} in early stage and study the relationship between \textit{H. pylori} infection and gastric carcinoma in order to decrease the incidence rate and prevent gastric carcinoma.

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