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World J Gastroenterol 2001;7(6):779-782
World Journal of Gastroenterology
 ISSN 1007-9327

• ORIGINAL RESEARCH •

Apoptosis, proliferation and *p*53 gene expression of *H. pylori* associated gastric epithelial lesions

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Received 2001-06-12 **Accepted** 2001-08-20

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 *p*53 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 superficiale pithelium and a few proliferative cells within the neck of gastric glands, and n o *p*53 protein expression in normal mucosa. In gastric carcinoma, there were few apoptotic cells, while there were a large number of proliferative cells, and expression of *p*53 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 *p*53 expression (46.7%) in *H.pylori*-positive group were higher than those in normal mucosa (*P*<0.01). AI in *H.pylori*-positive group w as 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 5%) 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 gradu 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 *p*53 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 *p*53 gene.**

Subject headings *Helicobacter pylori*; gastric precancerous lesion; apoptosis; proliferation; *p*53 gene

Zhang Z, Yuan Y, Gao H, Dong M, Wang L, Gong YH. Apoptosis, proliferation and *p*53 gene expression of *H. pylori* associated gastric epithelial lesions. *World J Gastroenterol*, 2001;7(6):779-782

INTRODUCTION

H. pylori infection is epidemiologically associated with the development of gastric cancer[1-7], 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 *p*53 gene expression in *H.pylori*-negative or *H.pylori*-positive gastric precan cerous lesion as well as gastric carcinomas. The purpose is to probe into the effect of *H.pylori* on apoptosis, proliferation and *p*53 gene expression in gastric epithelium and to find out the relationship between *H.pylori* and gastric carcinog enesis, 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[9-11] and PCR[12]. 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 xylem 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_2O_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℃ for 60 min; ⑥ added with STOP/WASF buffer at 37℃ 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 deh yd rated, transparency, mounted . PBS was substituted for TdT as negative control.

Immunohistochemical staining

SP kit was used (Zymed, USA).The primary a ntibodies were PCNA monoclonal antibody(diluted 1:50) and *p*53 monoclonal anti body (ready to use, Maixin, Fijian), 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

t test was used to compare the means. The positivity of *p*53 protein was analyzed by X^2 test

RESULTS

Charateristics 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 pro duct of *p*53 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 *p*53 protein was not seen. But in the tissue of gastric carcinoma, apoptotic cells accounted for 1.62%; prolifer a tive cells for 41.99% and a cluster was formed all over the lesions; and 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.

Table 1 Effect of *H. pylori* infection on AI in gastric epithelium (%, $\bar{x}\pm s$)

Group	*H.pylori* positive		*H.pylori* negative	
	n	AI	*n*	AI
Normal	-	-	20	2.08±1.07
Metaplasia	15	4.36±1.95[d]	15	3.81±1.76
Dysplasia	15	2.31±1.10[b]	15	3.05±1.29
Carcinoma	10	1.34±0.69[a]	10	1.89±1.03

[d]$P<0.01$, *vs* normal (*H.pylori* negative); [a] $P<0.05$, [b]$P<0.01$, *vs* upper adjacent 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*-posi tive 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 *p*53 protein expresion in *H.pylori*-positive group was higher than that in noraml mucosa ($P<0.01$). In the metaplasia,dysplasia mucosa and gastric carcinoma, there was a trend of gradual increase in positivity of *p*53 protein expression (Table 3) in *H. pylori*-positive group.

Table 2 Effect of *H.pylori* infection on PI in gastric epithelium (%, $\bar{x}\pm s$)

Group	*H.pylori* positive		*H.pylori* negative	
	n	PI	*n*	PI
Normal	-	-	20	9.78±3.65
Metaplasia	15	19.11±6.79[e]	15	12.25±5.63[d]
Dysplasia	15	33.89±11.65[eb]	15	22.09±8.18[d]
Carcinoma	10	48.27±15.67[ea]	10	34.70±12.74[c]

[e]$P<0.01$,*vs* normal (*H.pylori* negative); [a]$P<0.05$, [b]$P<0.01$,*vs* upper adjacent group; [c]$P<0.05$, [d]$P<0.01$, *vs* positive group.

Table 3 Effect of *H.pylori* infecion on expression of *p*53 protein in gastric epithelium

Group	*n*	*H.pylori*	*n*	*p*53 positive *n* (%)
Normal	20		20	0 (0.0)
Metaplasia	30	+	15	7 (46.7)[b]
		-	15	1 (6.7)
Dysplasia	30	+	15	8 (53.3)
		-	15	5 (33.3)
Carcinoma	20	+	10	8 (80.0)
		-	10	7 (70.0)

[b]$P<0.01$, *vs* normal (*H. pylori* negative).

DISCUSSION

Gastric mucosa consists of continuously renewed cells and cell proliferation an d apoptosis maintain their balance[13]. 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 prolife rating zone”. This distribution shows the proliferating zone gradually maturation, aging and death to the surface in the gastric mucosal epithelium. However in gastric carcinoma, apoptotic cells amount to 1.62%, proliferative cells amount t o 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[14-17]. 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 dysphasia mucosa, AI in *H.pylori*-positive group is lower than that in *H.pylori*-negative group. In the process of gastric carcinogenesis[14,15], 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 cytotixic protein (CagA and VacA)[20-28], and gastric mucosa can increasex some cytokines, nitrous oxide sythetase and oxygen radicals released after *H.pylori* infection[29-35]. 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[36],and this may be the mechanism that *H. pylori* in duces apoptosis. It has been proved that wild type *p*53 protein can induce cell apoptosis but the intracellular accumulation of mutant *p*53 protein can inhibit cell apoptosis and promote cell transformation and proliferation, resulting in carcinogenesis[37-41]. In this study, in the phae of metaplasia, positivity of *p*53 protein expression in *H.pylori*-positive group was higher than that in nor malmucosa. From the phase of metaplasia to gastric carcinoma, the positivity of *p*53 protein expression increased in *H. pylori*-positive group. As positive *p*53 protein confirmed by immunohistochemical staining was considered as mutation type[42]. It suggests that beginning with metaplasia, *H.pylori* infect ion can strengthen the expression of mutant *p*53 gene. With the accumulation of mutant and expression of *p*53, its inhibiting effect on apoptosis[43-45] will overpass the indution effect of *H. pylori*. Therefore in the *H.pylori*-positive dyspl asiamucosa, there was a decrease in apoptosis. In this study, in the *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 *H.pylori*-positive group. T here sult shows that *H.pylori* apparently improves gastric epithelium proliferation which is very similar with the proliferating characteristics of gastric carcinoma. The mechanism may be that urease enzyme produced by *H. pylori* hydrolyses urea into carbon dioxide and ammonia, the latter can promote mitosis[46]; *H. pylori* infection will increase mucosal content and expression of EGF and TGF-α[47-52] and the effect of mutant *p*53 on proliferation, will result in rapid proliferation of epithelium.

In the phase of metaplasia, *H.pylori* can induce apoptosis, stimulate hyperproliferation and result in the regulation disorder of 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 *p*53 protein, *H. pylori* induce s gastric epithelial cell hyperproliferation and apoptosis reduction or even imb alance of the apoptotic and proliferative process, and accumul at ion of DNA-damaged cells, ultimately resulting in gastric carcinogenesis.This makes it clear that *H. pylori* infection may be an important factor of gastric car cinogenesis. Thus, it is significant to prevent *H. pylori* infection, eradicate *H. pylori* in early stage and study the relationship between *H. pylori* infection and gastric carcinoma in order to decrease the incidence rate and prevent gastric carcinoma.

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Edited by Xu XQ and Wang JH Verified by Ma JY