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Effect of *Helicobacter pylori* infection on expressions of Bcl-2 family members in gastric adenocarcinoma

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Abstract

AIM: To investigate the effect of *Helicobacter pylori* (*H pylori*) infection on the expressions of Bcl-2 family members in gastric adenocarcinoma.

METHODS: Gastric adenocarcinoma and resection margin tissues of 95 patients were studied. Semi-quantitative RT-PCR was used to measure Bid, Bax and Bcl-2 mRNA expressions.

RESULTS: Expressions of Bid and Bax in gastric adenocarcinoma tissues without *H pylori* infection, with cagA$^-$ *H pylori* infection and cagA$^+$ *H pylori* infection increased significantly in turn (Bid, 0.304, 0.422 and 0.855 respectively, $P<0.05$; Bax, 0.309, 0.650 and 0.979 respectively, $P<0.05$). Bcl-2 mRNA levels increased significantly in gastric adenocarcinoma tissues with cagA$^-$ *H pylori* infection and cagA$^+$ *H pylori* infection, compared with those without *H pylori* infection (0.696 and 0.849 *vs* 0.411, $P<0.05$). Expressions of Bid, Bax and Bcl-2 in resection margin tissues without *H pylori* infection, with cagA$^-$ *H pylori* infection and cagA$^+$ *H pylori* infection increased significantly in turn (Bid, 0.377, 0.686 and 0.939 respectively, $P<0.05$; Bax, 0.353, 0.645 and 1.001 respectively, $P<0.05$; Bcl-2, 0.371, 0.487 and 0.619 respectively, $P<0.05$). In *H pylori* negative specimens, expressions of Bid and Bax correlated negatively with that of Bcl-2 respectively in adenocarcinoma tissues (Bid *vs* Bcl-2, $r=-0.409$, $P<0.05$; Bax *vs* Bcl-2, $r=-0.451$, $P<0.05$). In *H pylori* positive specimens, expressions of Bid and Bax did not correlate with that of Bcl-2 in adenocarcinoma tissues (Bid *vs* Bcl-2, $r=0.187$, $P>0.05$; Bax *vs* Bcl-2, $r=0.201$, $P>0.05$), but correlated positively with that of Bcl-2 respectively in resection margin tissues (Bid *vs* Bcl-2, $r=0.331$, $P<0.05$; Bax *vs* Bcl-2, $r=0.295$, $P<0.05$).

CONCLUSION: *H pylori* may enhance Bid, Bax and Bcl-2 mRNA levels and cause deregulation of these apoptosis-associated genes expressions, which may play a role during development of gastric adenocarcinoma induced by *H pylori*.

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INTRODUCTION

Helicobacter pylori (*H pylori*) infection is the most common chronic infection in humans and is the major cause of gastritis worldwide. This infection is also accepted as the etiological factor of the majority of peptic ulcers. It has been implicated as a significant contributing factor in the development of gastric malignancy, both gastric MALT lymphoma and gastric adenocarcinoma[1-14], and *H pylori* was classified as a group 1 carcinogen for gastric cancer in 1994 by the WHO and International Agency for Research on Cancer (IARC)[15]. The role of *H pylori* infection in the gastric carcinogenesis is not clear. It might be involved in imbalance between apoptosis and proliferation[16-33]. Bcl-2 family members have been closely related to apoptosis, which could either promote cell survival (Bcl-2, Bcl-x_L, A1, Mcl-1, and Bcl-w) or promote cell death (Bax, Bak, Bcl-x_S, Bad, Bid, Bik, Bim, Hrk, Bok)[34-36]. In the present study, we investigated the effect of *H pylori* infection on the expressions of Bcl-2 family members in gastric adenocarcinoma and resection margin tissues.

MATERIALS AND METHODS

Tissue specimens

Specimens of gastric adenocarcinoma of 95 patients (72 males and 23 females, age range 31 to 84 years, mean 56 years), who had undergone resection surgical at the Southwest Hospital in Chongqing and had not taken anti-*H pylori* drugs before operation, were collected from 2001 to 2002. Gastric adenocarcinoma tissues were examined microscopically. Resection margin tissues were also examined to verify that they did not contain malignant cells. The histological diagnosis was confirmed by a professional pathologist. The remaining specimens were snap-frozen and stored at -80 ℃ until assayed. Warthin-Starry silver staining and polymerase chain reaction (PCR) analysis for *H pylori* urease gene A (*ure*A) were performed to detect *H pylori* infection. PCR analysis for *H pylori cag*A gene was performed to verify *cag*A$^+$ *H pylori* infection. Fifty-eight patients whose both Warthin-Starry staining and PCR for *ure*A showed positive results were diagnosed as suffering from *H pylori* infection and 37 were *cag*A$^+$ *H pylori*.

RT-PCR analysis of Bid, Bax and Bcl-2 mRNA

According to references[37,38], primers were designed for β-actin (GenBank accession No.BC013380), 5' -GTG GGG CGC CCC AGG CAC CA-3' (sense) and 5' -CTC CTT AAT GTC ACG CAC GAT TTC-3' (antisense), 540 bp product; for Bid (GenBank accession No.AF087891), 5' -ATG GAC TGT TGA GGT CAA CAA C-3' (sense) and 5' -TCA GTC CAT CCC ATT TCT GGC T-3' (antisense), 588 bp product; for Bax (GenBank accession No.AY217036), 5' -ACC AAG AAG CTG AGC GAG TGT C-3' (sense) and 5' -ACA AAG ATG GTC ACG GTC TGC C-3' (antisense), 332 bp product; and for Bcl-2 (GenBank accession No.M13994), 5' -TGC ACC TGA CGC CCT TCA C-3' (sense), 5' -AGA CAG CCA GGA GAA ATC AAA CAG-3' (antisense), 293 bp product.

Total RNA was prepared from gastric adenocarcinoma

tissues and resection margin tissues by using TriPure isolation reagent (Roche) according to the manufacturer's protocol. Reverse transcription (RT) was performed for first-strand cDNA by using 2 μg of total RNA and 1 μl of oligo(dT)18 primer in the presence of 5 unit AMV reverse transcriptase (Promega), 20 unit RNase inhibitor, 0.5 mmol/L of each dNTP and 1×buffer in 20 μl for 60 min at 42 ℃. Then 2 μl reverse transcription products were used for PCR. In a total of 20 μl reactive mixture, 5 pmol/L sense primer and 5 pmol/L antisense primer, 0.25 mmol/L of each dNTP, 1×reaction buffer and 1.5 unit Taq polymerase were mixed. The reaction was run for 33 cycles, and each consisted of denaturation at 94 ℃ for 60 s, annealing at 58 ℃ for 60 s, extension at 72 ℃ for 60 s and final extension prolonged for 7 min at 72 ℃. PCR-amplified products (8 μl each) were analyzed on 1.5% agarose gels after ethidium bromide staining. Expression levels of Bid, Bax and Bcl-2 were quantitated using Quantity One quantitation software (Bio-Rad Laboratories) and were reported to be normalized to β-actin levels.

Statistical analysis

All data were presented as means ± standard error. Differences in means were examined by ANOVA, and correlations were analyzed by using Spearman's rank correlation coefficient (SPSS 10.0 for Windows). A P value<0.05 was considered significant.

RESULTS

Effect of H pylori on expressions of Bid, Bax and Bcl-2 mRNA

In gastric adenocarcinoma tissues, expressions of Bid and Bax mRNA in *H pylori* negative group, cagA$^-$ *H pylori* infection group and cagA$^+$ *H pylori* infection group increased in an ascending pattern, respectively (P<0.05). Expression of Bcl-2 in *H pylori* negative group was significantly lower than that in *H pylori* infection group (P<0.05). Levels of Bcl-2 mRNA between cagA$^-$ *H pylori* infection group and cagA$^+$ *H pylori* infection group did not show any significant difference.

In resection margin tissues, expressions of Bid, Bax and Bcl-2 mRNA in *H pylori* negative group, cagA$^-$ *H pylori* infection group and cagA$^+$ *H pylori* infection group increased in turn (P<0.05) (Figure 1, Tables 1-3).

Table 1 Effect of *H pylori* infection on expression of Bid

		Adenocarcinoma	Resection margin
H pylori (-)		0.304±0.113	0.377±0.119
H pylori (+)	cagA (-)	0.422±0.149[a]	0.686±0.285[a]
	cagA (+)	0.855±0.305[ac]	0.939±0.383[ac]

[a]P<0.05, *vs H pylori* negative group, [c]P<0.05, *vs* cagA$^-$ *H pylori* infection group.

Table 2 Effect of *H pylori* infection on expression of Bax

		Adenocarcinoma	Resection margin
H pylori (-)		0.309±0.123	0.353±0.139
H pylori (+)	cagA (-)	0.650±0.393[a]	0.645±0.327[a]
	cagA (+)	0.979±0.375[ac]	1.001±0.361[ac]

[a]P<0.05, *vs H pylori* negative group, [c]P<0.05, *vs* cagA$^-$ *H pylori* infection group.

Table 3 Effect of *H pylori* infection on expression of Bcl-2

		Adenocarcinoma	Resection margin
H pylori (-)		0.411±0132	0.371±0.153
H pylori (+)	cagA (-)	0.696±0.318[a]	0.487±0.241[a]
	cagA (+)	0.849±0.352[a]	0.619±0.243[ac]

[a]P<0.05, *vs H pylori* negative group, [c]P<0.05, *vs* cagA$^-$ *H pylori* infection group.

Correlation among levels of Bid, Bax and Bcl-2 mRNA

In *H pylori* negative group, levels of Bid and Bax mRNA correlated negatively with that of Bcl-2 in gastric adenocarcinoma tissues (Bid *vs* Bcl-2,r=-0.409, P<0.05; Bax *vs* Bcl-2, r=-0.451, P<0.05). In *H pylori* positive group, expressions of Bid and Bax did not correlate with that of Bcl-2 in adenocarcinoma tissues (Bid *vs* Bcl-2, r=0.187, P>0.05; Bax *vs* Bcl-2, r=0.201, P>0.05), but correlated positively with that of Bcl-2 respectively in resection margin tissues (Bid *vs* Bcl-2, r=0.331, P<0.05; Bax *vs* Bcl-2, r=0.295, P<0.05).

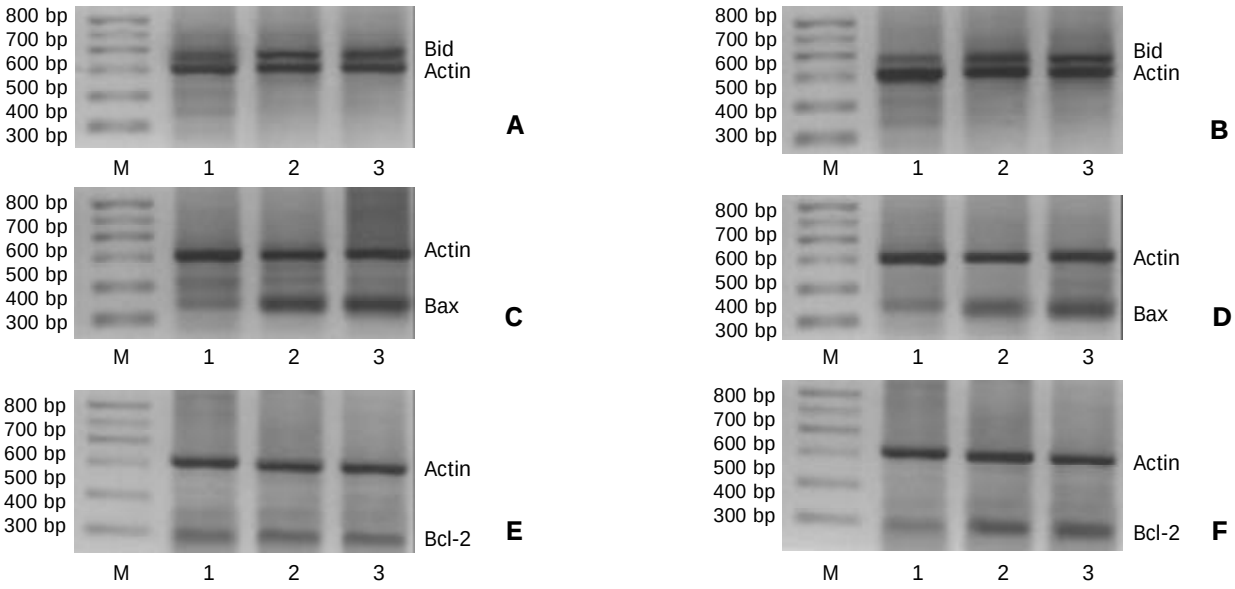

Figure 1 Effect of *H pylori* infection on mRNA expressions of Bcl-2 family members in gastric adenocarcinoma and resection margin tissues. A: Expression of Bid in gastric adenocarcinoma, B: Expression of Bid in resection margin, C: Expression of Bax in gastric adenocarcinoma, D: Expression of Bax in resection margin, E: Expression of Bcl-2 in gastric adenocarcinoma, F: Expression of Bcl-2 in resection margin, 1: *H pylori* negative group, 2: cagA$^-$ *H pylori* group, 3: cagA$^+$ *H pylori* group.

DISCUSSION

Bid, Bax and Bcl-2 are representative members of Bcl-2 family. Bid and Bax are pro-apoptosis members. Bcl-2 is an anti-apoptosis member. In the mechanism of regulating apoptosis, Bid as a BH3 domain protein, is one of the initiators to apoptosis and Bax is the key member. Either Bid or Bcl-2 must rely on Bax to induce or inhibit apoptosis[39-43].

Studies have shown that *H pylori* infection could induce Fas antigen (Fas Ag) expression in gastric epithelial cells[44]. In addition, *H pylori* infection was also associated with increased mucosal inflammatory cytokines, including TNF-α[45] and IFN-γ[46]. The cytokines generated during the immune response to *H pylori* also increased expression of Fas Ag in gastric cell lines[47]. Fas Ag, after binding specifically to its ligand (Fas L), trimerizes and activates Caspase-8. Activation of Caspase-8 could result in the cleavage of cytosolic Bid to truncate tBID, which could translocate to mitochondria and initiate apoptosis[48]. Shibayama *et al*[31] found that *H pylori* infection induced the activation of Caspase-8 and the expression of Bid in human gastric epithelial cells, and inhibition of Caspase-8 suppressed the expression of Bid. In the present study, we found that *H pylori* infection upregulated expression of Bid mRNA in both gastric adenocarcinoma and resection margin tissues. That might be due to the upregulation of Fas and the activation of Caspase-8.

Bax is a cytosolic protein and translocates from the cytosol to the mitochondria for integration into the membrane following a proapoptotic stimulus. This action then results in cytochrome C release and initiates apoptosis. We have previously demonstrated that *H pylori* infection could promote Bax protein expression in chronic gastritis and premalignant lesions. Expression of Bax correlated positively with apoptotic index. The apoptotic index in Bax expression positive group in intestinal metaplasia, gastric dysplasia and gastric carcinoma was significantly higher than that in Bax negative group. In the present study, we found *H pylori* infection also increased levels of Bax mRNA in both gastric adenocarcinoma and resection margin tissues, and the effect was stronger in $CagA^+$ *H pylori* group than $cagA^-$ *H pylori* group. These results showed *H pylori* infection might promote apoptosis in gastric adenocarcinoma and its resection margin tissues.

Bc1-2 is an important anti-apoptosis protein. We found that Bcl-2 mRNA levels in *H pylori* negative group in gastric adenocarcinoma tissues were lower than in *H pylori* positive group. In resection margin tissues, Bcl-2 mRNA levels in *H pylori* negative group, $cagA^-$ *H pylori* infection group and $cagA^+$ *H pylori* infection group respectively increased in turn, suggesting that *H pylori* might promote expression of Bcl-2 in both gastric adenocarcinoma and resection margin tissues.

Although *H pylori* could promote expressions of Bid, Bax and Bcl-2, the correlations among them are still unclear. In the present study, it showed that levels of Bid and Bax mRNA were correlated negatively with that of Bcl-2 in gastric adenocarcinoma tissues without *H pylori* infection. This result is correspondent with the findings that apoptosis decreases in tumor tissues. In *H pylori* positive group, levels of Bid, Bax and Bcl-2 mRNA in gastric adenocarcinoma all increased. Besides, levels of Bid, Bax, and Bcl-2 did not correlate with each other. In the resection margin tissues, levels of Bid and Bax mRNA were correlated positively with that of Bcl-2. These results indicated that although *H pylori* could promote expressions of Bid, Bax and Bcl-2, it might play a different role in the development of gastric adenocarcinoma. In benign gastric lesions, *H pylori* infection might mainly upregulate expressions of pro-apoptotic genes such as Bid and Bax, and this effect might be stronger than its upregulatory effect on Bcl-2, which is consistent with the phenomena that *H pylori* infection increases apoptosis in atrophic gastritis and gastric ulcer. During development of gastric adenocarcinoma, upregulatory effect of *H pylori* on anti-apoptotic genes, for example Bcl-2, might increase gradually and counteract pro-apoptosis effect of Bid and Bax, which may induce or worsen deregulation of apoptosis-associated genes expressions during the course of the formation of gastric adenocarcinoma.

Recently, it was found that not only excessive proliferation played an important role in gastric adenocarcinoma, but also reduction of apoptosis contributed to the carcinogenesis of gastric mucosa. The abnormal expressions of Bid, Bax and Bcl-2 induced by *H pylori* might result in inhibition of apoptosis, which may play an important role during development of gastric adenocarcinoma induced by *H pylori*[49]. The detailed mechanism still remains to be studied.

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