ORIGINAL ARTICLE

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Helicobacter pylori Eradication Downregulates Cellular Inhibitor of Apoptosis Protein 2 in Gastric Carcinogenesis

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Background/Aims: To evaluate the expression of cellular inhibitor of apoptosis protein 2 (*cIAP2*) during gastric carcinogenesis after *Helicobacter pylori* (HP) infection and after HP eradication. **Methods:** We divided non-cancer patients into four groups according to the status of HP infection and atrophic gastritis (AG)/intestinal metaplasia (IM). We compared *cIAP2* mRNA expression among these four groups and patients with HP-positive early gastric cancer (EGC) by using real-time polymerase chain reaction (PCR). We evaluated the expression of *cIAP2* messenger RNA (mRNA)/protein by using real-time PCR/immunohistochemistry and the degree of apoptosis with a terminal deoxynucleotidyl transferase-mediated nick end labeling assay before and 12 months after endoscopic submucosal dissection (ESD) in HP-positive EGC patients, regardless of whether they had undergone eradication therapy. **Results:** The expression of *cIAP2* mRNA was significantly higher in the groups with HP(+), AG/IM(+), and HP-positive EGC than in the control, HP(+), and AG/IM(−) groups ($p<0.005$). In the HP eradication group, the expression of *cIAP2* mRNA/protein significantly decreased ($p=0.006$) and apoptosis increased at the 12-month follow-up after ESD. In the HP noneradication group, the aforementioned changes were not found during the same follow-up period. **Conclusions:** The expression of *cIAP2* increased during gastric carcinogenesis after HP infection; HP eradication in the patients who had undergone ESD for EGC reversed overexpression of *cIAP2* and suppressed cell apoptosis. **(Gut Liver 2017;11:79-86)**

Key Words: *Helicobacter pylori*; Cellular inhibitor of apoptosis protein 2; Gastric carcinogenesis

INTRODUCTION

Inhibitor of apoptosis protein (IAP) is defined as a protein containing one or more repeats of the baculovirus IAP domain.[1] IAP is one of the major proteins controlling apoptosis, which plays an important role in both normal development and diseases. Until now, eight types of IAP family proteins have been identified. Among these, cellular inhibitor of apoptosis protein 2 (*cIAP2*) is known to play an important role in carcinogenesis.[2] *cIAP2* is induced by nuclear factor-κB (NF-κB),[3] and is reported to inhibit apoptosis by downregulating certain caspases.[4] Overexpression of *cIAP2* has been reported in colon and pancreatic cancer.[5,6] However, studies investigating the role of *cIAP2* in gastric carcinogenesis are scarce.

Causing an imbalance between cell proliferation and apoptosis is one of several mechanisms through which *Helicobacter pylori* induces gastric cancer.[7,8] *H. pylori* is known to either activate or inhibit apoptosis depending on the degree, duration, and nature of infection. For example, *H. pylori* expressing *CagA* and *VacA* stimulates apoptosis in gastric epithelial cells.[9] Proapoptotic genes were overexpressed in *H. pylori*-infected patients,[10] and apoptosis increased in the early stage of gastric carcinogenesis due to *H. pylori* infection.[11] Conversely, it was also reported that *H. pylori* can inhibit apoptosis. It is known that *H. pylori* activates NF-κB in a *Cag* pathogenicity island-dependent manner and subsequently induces the up-regulation of *cIAP2* expression.[7,12] Expression of both NF-κB and *cIAP2* increased when gastric cancer cell lines were cocultured with *CagA*-positive *H. pylori*.[12] Conversely, knockdown of *cIAP2* in gastric cancer cell lines resulted in increased apoptosis, decreased cell proliferation, and delayed cell migration.[13] In mouse models, *H.*

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pylori infection induced increased *cIAP2* in the gastric mucosa.[13] Finally, *cIAP2* was reportedly overexpressed in more than 70% of human gastric cancer tissues;[13] overexpression was more prominent in cancer tissues than in adjacent non-cancer tissues, and in *H. pylori*-positive patients than in *H. pylori*-negative patients.[14] Therefore, it was suggested that *cIAP2* overexpression plays an important role in *H. pylori*-induced gastric carcinogenesis. However, the exact stage at which overexpression of *cIAP2* commences during progression from *H. pylori* infection to gastric cancer is unclear.

The first aim of this study was to evaluate *cIAP2* overexpression during human gastric carcinogenesis after *H. pylori* infection in the context of atrophic gastritis (AG) and intestinal metaplasia (IM). The second aim was to determine whether eradication of *H. pylori* in patients who underwent endoscopic submucosal dissection (ESD) for early gastric cancer (EGC) reverses *cIAP2* overexpression.

MATERIALS AND METHODS

1. Subjects and study design

This study was carried out in three stages. First, we determined the expression of several IAP family genes, including *cIAP2*, in the gastric mucosal samples of control subjects who were *H. pylori*-negative and showed no evidence of AG and IM in either the antrum or the body, and in patients with *H. pylori*-positive EGC. In the second step, we divided non-cancer patients into four groups according to the presence or absence of *H. pylori* infection and AG/IM, and we compared the expression of *cIAP2* mRNA among them as well as among *H. pylori*-positive EGC patients by real-time polymerase chain reaction (PCR). Lastly, we evaluated the expression of cIAP2 mRNA/protein and the degree of apoptosis before and 12 months after ESD in *H. pylori*-positive EGC patients irrespective of whether they received eradication therapy. In this stage, the expression levels of *cIAP2* protein and the degree of apoptosis were analyzed by immunohistochemistry (IHC) and terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay, respectively.

In the present study, gastric tissues of patients without EGC were acquired from subjects who underwent esophagogastroduodenoscopy (EGD) and were healthy or diagnosed with gastritis between October 2005 and October 2006. Gastric tissues of EGC patients were acquired from samples that were previously collected for another study; the summary of that study is as follows.[15] *H. pylori*-positive patients who underwent ESD for EGC were randomly assigned into eradication or noneradication group. Patients in the eradication group received 20 mg omeprazole, 1 g amoxicillin, and 500 mg clarithromycin twice daily for 1 week. Patients in the noneradication group received no antibiotics. All patients underwent follow-up EGD regularly for several years. Successful eradication was confirmed in the eradication group by both histologic evaluation and rapid urease test. The primary outcome was development of metachronous gastric carcinoma. We used some samples from that study to evaluate the effect of *H. pylori* eradication on cIAP2 expression and apoptosis in the EGC patients before and 12 months after ESD. This study was approved by the Institutional Review Board of Seoul National University Hospital (IRB number: H-1205-016-408).

2. Gastric tissue collection

We collected two sections of gastric mucosal tissue from the lesser curvature of the antrum and body by endoscopic biopsy from all subjects; in patients with EGC, noncancerous tissues were collected. *H. pylori* infection status was determined by histologic evaluation and the rapid urease test, which was performed in another tissue sample from the lesser curvature of the antrum. *H. pylori* infection was deemed positive if at least one of two tests was positive. The degree of AG and IM in the gastric mucosa was classified according to the updated Sydney system.[16] Negative AG/IM was defined as no evidence of AG and IM in both the antrum and body. Positive AG/IM was defined as moderate or high AG or IM in either the antrum or body.

3. Real-time PCR microarray for IAP family genes

We collected four samples each from control subjects who were *H. pylori*-negative and showed no AG and IM, and from subjects with *H. pylori*-positive EGC. We then extracted the total RNA and performed quality control tests; two samples from each group were selected. Briefly, total RNA was extracted using the RNeasy midi kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and treated with RNase-free DNase I (Promega, Madison, WI, USA) to reduce DNA contamination. Total RNA concentration and purity were determined by measuring the 260:280 nm absorbance ratio using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The integrity of RNA samples was confirmed by the appearance of distinct 28S and 18S bands of ribosomal RNA using a Bioanalyzer 2100 system (Agilent Technology, Santa Clara, CA, USA).

We then analyzed mRNA expression of several IAP family genes including *cIAP2* by real-time PCR microarray. Briefly, complementary DNA (cDNA) was synthesized from 1 μg total RNA according to the manufacturer's instructions (RT2 First Strand kit; Qiagen). For quantitative comparison of mRNA levels, real-time PCR was performed using Human Apoptosis RT^2 Profiler™ PCR Array (Qiagen Korea Ltd., Seoul, Korea). This real-time PCR microarray consisted of a 96-well plate and included 84 genes closely related to apoptosis and cell survival, in addition to positive/negative controls. Two independent assays were performed for each condition. Gene expression was measured relative to the mean expression of β-actin as the endogenous control. Reactions were carried out on a StepOnePlus™ Real-

Time PCR System (Applied Biosystems, Foster City, CA, USA). Real-time PCR detection was performed under the following thermal cycling conditions: 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds and 60°C for 1 minutes. For analysis using the ΔΔCt method, we used the PCR Array Data Analysis Software (SaBiosciences; http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php).

4. Real-time PCR for *cIAP2* mRNA

Gastric mucosal specimens for real-time PCR were collected from the lesser curvature of the antrum. Total RNA was extracted from the specimens using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) per manufacturer's instructions and purified using RNeasy mini kits (Qiagen, Valencia, CA, USA).[17] RNA samples were then treated with DNase I (Invitrogen) and quantified by spectrophotometry. Total RNA was amplified and then labeled in the presence of fluorescent dNTP (Cy3 dUTP or Cy5 dUTP; GE Healthcare, Piscataway, NJ, USA). RNA samples were diluted to a final concentration of 0.5 mg/mL in RNase-free water and stored at -80°C until use. cDNA synthesis was performed with 1 mg of total RNA with M-MLV reverse transcription reagents (Invitrogen), and real-time PCR was conducted using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems) in 20 μL TaqMan Gene Expression Master Mix (Applied Biosystems) using 200 ng cDNA. The primary sequences used for *cIAP2* were as follows: forward, 5′-TCCTAGCTGCAGATTCGTTC-3′; reverse, 5′-CAAAGCAAGCCACTCTGTCT-3′ for a 349 bp PCR product. The primary sequences of GAPDH, which was used for the internal control, were as follows: forward, 5′-GCTCTCTGCTCCTCCTGTTC-3′; reverse, 5′-CGACCAAATCCGTTGACTCC-3′ for a 114 bp PCR product. The reaction conditions were 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The human GAPDH gene was used as an endogenous reference when calculating Ct values. Relative gene expression values were determined from the Ct values using the $2^{-\Delta\Delta Ct}$ method.[18]

5. IHC for *cIAP2* protein

Tissues from the antrum were fixed in 10% neutral buffered formalin, paraffin-embedded, and then cut into 4-μm sections. Slides were stained using the Discovery XT automated immunohistochemistry stainer (Ventana Medical Systems Inc., Tucson, AZ, USA) and detection was performed using the Ventana Chromo Map Kit (Ventana Medical Systems). Sections were deparaffinized using the EZ Prep solution. CC1 (pH 8.4 buffer containing Tris/Borate/EDTA) was used for antigen retrieval. Endogenous peroxidases were blocked with Inhibitor D (3% H_2O_2) for 4 minutes at 37°C temperature. Slides were incubated with primary antibodies for 32 minutes at 37°C and a secondary antibody for 20 minutes at 37°C. Slides were incubated in diaminobenzidine (DAB) plus H_2O_2 substrate for 8 minutes at 37°C followed by hematoxylin and bluing reagent counterstain at 37°C. Reaction buffer (pH 7.6 Tris buffer) was used as a washing solution. A monoclonal antibody for cIAP2 (Santa Cruz Biotechnology; Santa Cruz, CA, USA; dilution 1:30) was used as a primary antibody, and biotin-labeled anti-mouse immunoglobulin G (UltraMap anti-Ms HRP Roche; Ventana Medical Systems, Inc.) was used as a secondary antibody. Negative controls were treated similarly while excluding the primary antibodies. The whole slide area was assessed and positive staining was qualitatively evaluated by an experienced pathologist (E.S.).

6. TUNEL assay

To evaluate apoptosis, TUNEL staining was performed using the Apoptag® Peroxidase In Situ Apoptosis Detection Kit S7100 (Millipore Corp., Billerica, MA, USA).[19] After deparaffinization with xylene and graded concentrations of alcohol, gastric mucosal tissue sections were exposed to Proteinase K for 15 minutes at room temperature. Endogenous peroxidase activity was quenched with Inhibitor D for 5 minutes at room temperature. Sections were incubated with terminal deoxynucleotidyl transferase (TdT) in a humidified chamber at 37°C for 1 hour. After incubation with anti-digoxigenin-conjugate for 30 minutes at room temperature, peroxidase substrate and 0.05% DAB was applied to develop color. The specimens were then washed with distilled water and counterstained with 0.5% methyl green for 10 minutes at room temperature. Sections were counterstained with Mayer's hematoxylin. Identically-treated slides not exposed to TdT served as negative controls. In all cases, positive staining was qualitatively evaluated on the entire slide by an experienced pathologist (E.S.).

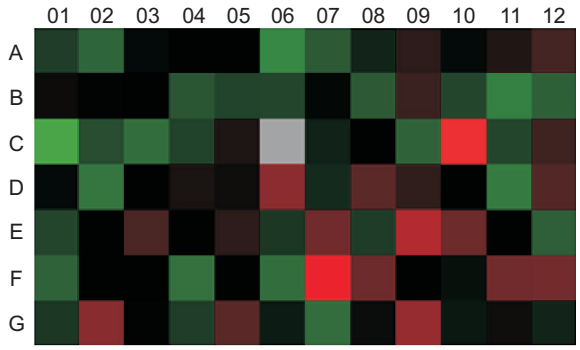
7. Statistical analysis

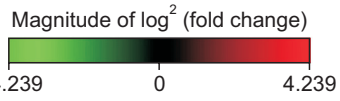
SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Because the data were not normally distributed (as demonstrated by the Shapiro-Wilk test), the relative expression of *cIAP2* mRNA among several groups was compared using a Kruskal-Wallis test. *Post hoc* comparisons of pairwise differences between two groups were evaluated by the Mann-Whitney U test using the modified Bonferroni procedure for multiple comparison adjustment. To compare the expression levels of *cIAP2* mRNA in *H. pylori*-positive EGC before and at 12 months after ESD, a Wilcoxon signed-rank test was performed. Results with p-values less than 0.05 were considered significant.

RESULTS

1. Real-time PCR microarray for IAP family genes

Fig. 1 shows the heat map of PCR microarray. Among the five IAP family genes analyzed (*Survive*, *cIAP1*, *cIAP2*, *XIAP*, and *NAIP*), only the *cIAP2* (BIRC3) mRNA was 1.85 times higher in subjects with *H. pylori*-positive EGC than in *H. pylori*-negative controls without AG/IM.





Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	ABL1 −1.67 OKAY	AIFM1 −2.32 OKAY	AKT1 −1.20 OKAY	APAF1 −1.03 OKAY	BAD 1.10 OKAY	BAG1 −3.28 OKAY	BAG3 −2.11 OKAY	BAK1 −1.39 OKAY	BAX 1.69 OKAY	BCL10 −1.20 OKAY	BCL2 1.58 A	BCL2A1 2.01 OKAY
B	BCL2L1 1.34 OKAY	BCL2L10 1.24 B	BCL2L11 1.01 A	BCL2L2 −2.01 OKAY	BFAR −1.74 OKAY	BID −1.78 OKAY	BIK −1.17 OKAY	BIRC2 −2.09 OKAY	BIRC3 1.85* OKAY	BIRC5 −1.78 OKAY	BIRC6 −3.06 OKAY	BNIP2 −2.26 OKAY
C	BNIP3 −4.69 OKAY	BNIP3L −1.86 OKAY	BRAF −2.57 OKAY	CASP1 −1.72 OKAY	CASP10 1.57 OKAY	CASP14 −4.46 C	CASP2 −1.36 OKAY	CASP3 −1.05 OKAY	CASP4 −2.29 OKAY	CASP5 18.89 A	CASP6 −1.76 OKAY	CASP7 1.89 OKAY
D	CASP8 −1.20 OKAY	CASP9 −2.78 OKAY	CD27 1.11 B	CD40 1.51 OKAY	CD40LG 1.40 A	CD70 3.50 A	CFLAR −1.46 OKAY	CIDEA 2.35 B	CIDEB 1.80 OKAY	CRADD 1.15 OKAY	CYCS −2.89 OKAY	DAPK1 2.18 A
E	DFFA −1.78 OKAY	DIABLO 1.05 OKAY	FADD 2.08 OKAY	FAS 1.14 OKAY	FASLG 1.70 A	GADD45A −1.57 OKAY	HRK 2.76 B	IGF1R −1.62 OKAY	IL10 5.24 A	LTA 2.69 B	LTBR 1.10 OKAY	MCL1 −2.18 OKAY
F	NA1P −2.28 OKAY	NFKB1 1.01 OKAY	NOD1 −1.08 OKAY	NOL3 −2.64 OKAY	PYCARD 1.19 OKAY	RIPK2 −2.56 OKAY	TNF 12.77 A	TNFRSF10A 2.69 A	TNFRSF10B 1.19 OKAY	TNFRSF11B −1.24 OKAY	TNFRSF1A 2.77 OKAY	TNFRSF1B 2.79 A
G	TNFRSF21 −1.55 OKAY	TNFRSF25 3.30 A	TNFRSF9 1.24 A	TNFSF10 −1.64 OKAY	TNFSF8 2.29 A	TP53 −1.33 OKAY	TP53BP2 −2.49 OKAY	TP73 1.37 B	TRADD 3.77 A	TRAF2 −1.29 OKAY	TRAF3 1.41 OKAY	XIAP −1.37 OKAY

Fig. 1. Heat map of the Human Apoptosis RT^2 Profiler^{TM} PCR Array. The gene symbol, value of log^2 fold change of mRNA expression, and reliability of the data are presented at the bottom. OKAY, A, and B indicate good, moderate, and poor data reliability, respectively. In the top grid, the magnitude of the log^2 fold change in mRNA expression of each gene is represented by the color of each square. Red indicates mRNA overexpression, and green indicates reduced mRNA expression. *Among the 5 IAP family genes analyzed (*Survive*, *cIAP1*, *cIAP2*, *XIAP*, and *NAIP*), the expression of only *cIAP2* (BIRC3) mRNA increased (by 1.85-fold) in subjects with *H. pylori*-positive early gastric cancer compared with the expression in *H. pylori*-negative controls without atrophic gastritis or intestinal metaplasia (row C, column 9).

2. Real-time PCR for *cIAP2* mRNA

We performed *cIAP2* mRNA real-time PCR for 22 samples in each group. However, the results of several samples were not interpretable, and were thus excluded. Therefore, the number of samples in each group varied, ranging between 18 and 22 (normal control, both *H. pylori* and AG/IM-positive group; *H. pylori*-positive EGC, n=22; *H. pylori*-positive and AG/IM-negative group, n=18; *H. pylori*-negative and AG/IM-positive group, n=20). Fig. 2 shows the expression levels of *cIAP2* mRNA in gastric mucosal tissues by real-time PCR. There was a significant difference in the relative expression of *cIAP* mRNA among the five groups (p<0.001). *Post hoc* analysis showed that the expression of *cIAP2* mRNA was significantly higher in patients positive for both *H. pylori* and AG/IM as well as in *H. pylori*-positive EGC patients than in control patients and those positive for *H. pylori* but negative for AG/IM (p<0.005).

3. Effect of *H. pylori* eradication on *cIAP2* expression and apoptosis

The effect *H. pylori* eradication on cIAP2 expression and apoptosis in the EGC patients after ESD was evaluated in 27 subjects (eradication group, n=12; noneradication group, n=15). Fig. 3 shows the expression of *cIAP2* mRNA by real-time PCR in patients with *H. pylori*-positive EGC before ESD and at 12 months following the procedure. The *cIAP2* mRNA levels before ESD were not significantly different between the eradication and noneradication groups (p=0.354). In the eradication group, the expression levels of *cIAP2* mRNA had decreased significantly after 12 months (p=0.006) (Fig. 3A). In the noneradication group, however, there was no difference in the expression of *cIAP2* mRNA before and 12 months after ESD (p=0.14) (Fig. 3B). Fig. 4 shows representative IHC staining for *cIAP2* protein in patients with *H. pylori*-positive EGC before ESD and at 12 months following the procedure. In the eradication group, the expression of *cIAP2* protein decreased at the 12-month follow-

up time (Fig. 4B). In the noneradication group, there was no difference in the expression of *cIAP2* protein before ESD and 12 months after (Fig. 4D). Fig. 5 shows representative TUNEL staining in patients with *H. pylori*-positive EGC before and at 12 months after ESD. In the eradication group, cell apoptosis had increased 12 months following the ESD procedure (Fig. 5B). In the noneradication group, there was no difference in cell apoptosis before and 12 months after ESD (Fig. 5D).

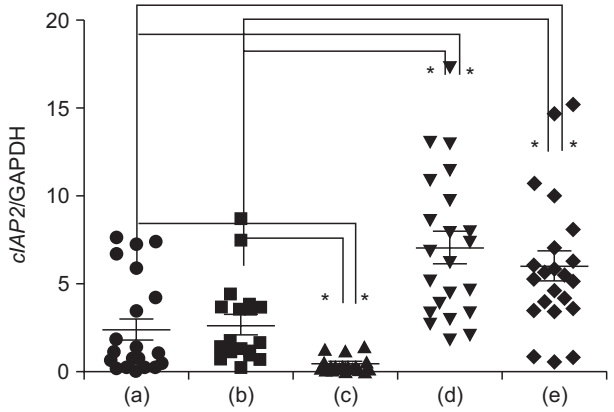


Fig. 2. Expression of *cIAP2* mRNA detected by real-time polymerase chain reaction in five groups classified according to the status of *Helicobacter pylori* infection, atrophic gastritis/intestinal metaplasia, and gastric cancer. There was a significant difference in the relative expression of *cIAP* mRNA among the five groups (p<0.001). (a) Normal control, (b) *H. pylori* (+) and AG/IM (–), (c) *H. pylori* (-) and AG/IM (+), (d) *H. pylori* (+) and AG/IM (+), (e) *H. pylori*-positive EGC.
AG, atrophic gastritis; IM, intestinal metaplasia; EGC, early gastric cancer. **Post hoc* analysis showed that the expression of *cIAP2* was significantly higher in the groups with *H. pylori* (+), AG/IM (+) (d) and *H. pylori*-positive EGC (e) than in the controls (a) (p<0.005).

DISCUSSION

Previous investigations of the relationship between *H. pylori* infection and *cIAP2* expression during gastric carcinogenesis were performed in gastric cancer cell lines cocultured with *H. pylori*[7,12] or in a mouse model of *H. pylori* infection.[13] In this study, we explored the role of *cIAP2* in *H. pylori*-induced gastric carcinogenesis in humans. We found that overexpression of *cIAP2* during gastric carcinogenesis requires not only *H. pylori* infection, but also progression to AG/IM. These results are consistent with previous reports showing that *H. pylori* has both apoptotic and antiapoptotic effects.[7] When *H. pylori* infection becomes chronic, antiapoptotic signaling pathways becomes predominant.[14] Moreover, the expression of *cIAP2* mRNA mildly decreased in patients with negative *H. pylori* infection and positive AG/IM compared to control patients. This indicates that progression of AG/IM is not sufficient to induce overexpression of *cIAP2* mRNA in gastric carcinogenesis; *H. pylori* infection is also required. Although we could not perform additional experiments to clarify the ring connecting *H. pylori* infection, AG/IM, and expression of *cIAP2*, we postulate that activation of NF-κB by *CagA* may be the primary mechanism for the development of *H. pylori*-specific gastric carcinogenesis.

Several studies reported that eradication of *H. pylori* after ESD in patients with EGC decreases the incidence of metachronous gastric cancer.[20-22] However, the underlying molecular mechanism has not been widely explored. Tsai *et al*.[23] reported that several genes related to cell proliferation were down-regulated in the gastric mucosa 1 year after *H. pylori* eradication. In the present study, we found that *cIAP2* overexpression and apoptosis suppression could be reversed within 1 year after *H. pylori* eradication in patients who underwent ESD for EGC. Taken together, our results suggest that risk reduction of metachronous

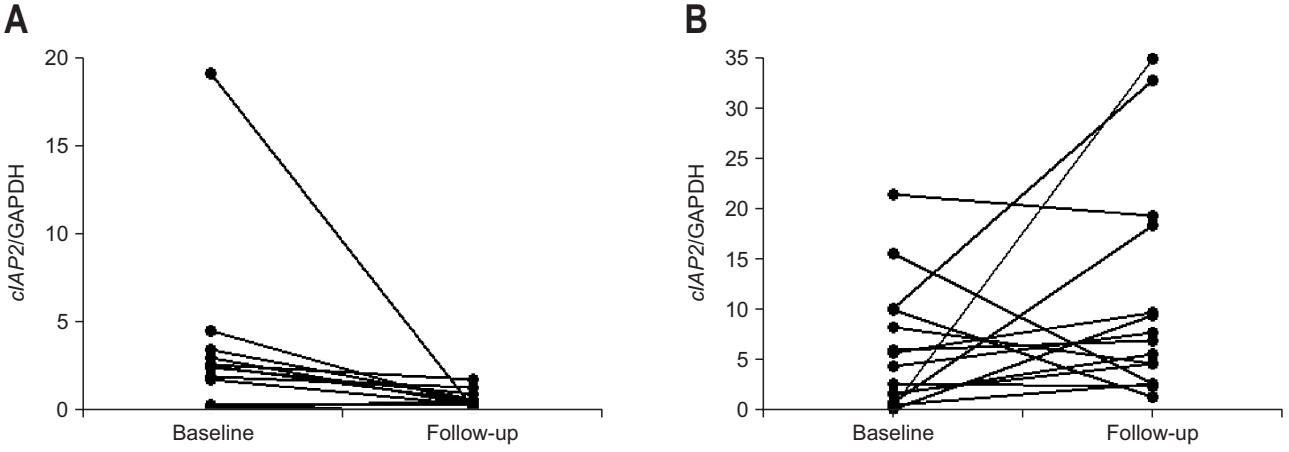


Fig. 3. The expression of *cIAP2* mRNA detected by real-time PCR in patients with *Helicobacter pylori*-positive early gastric cancer before endoscopic submucosal dissection and at the 12-month follow-up: (A) eradication group; (B) noneradication group. In the eradication group, the expression of *cIAP2* mRNA decreased significantly at the 12-month follow-up time point (p=0.006). In the noneradication group, there was no difference in the expression of *cIAP2* mRNA before and 12 months after endoscopic submucosal dissection (p=0.14).

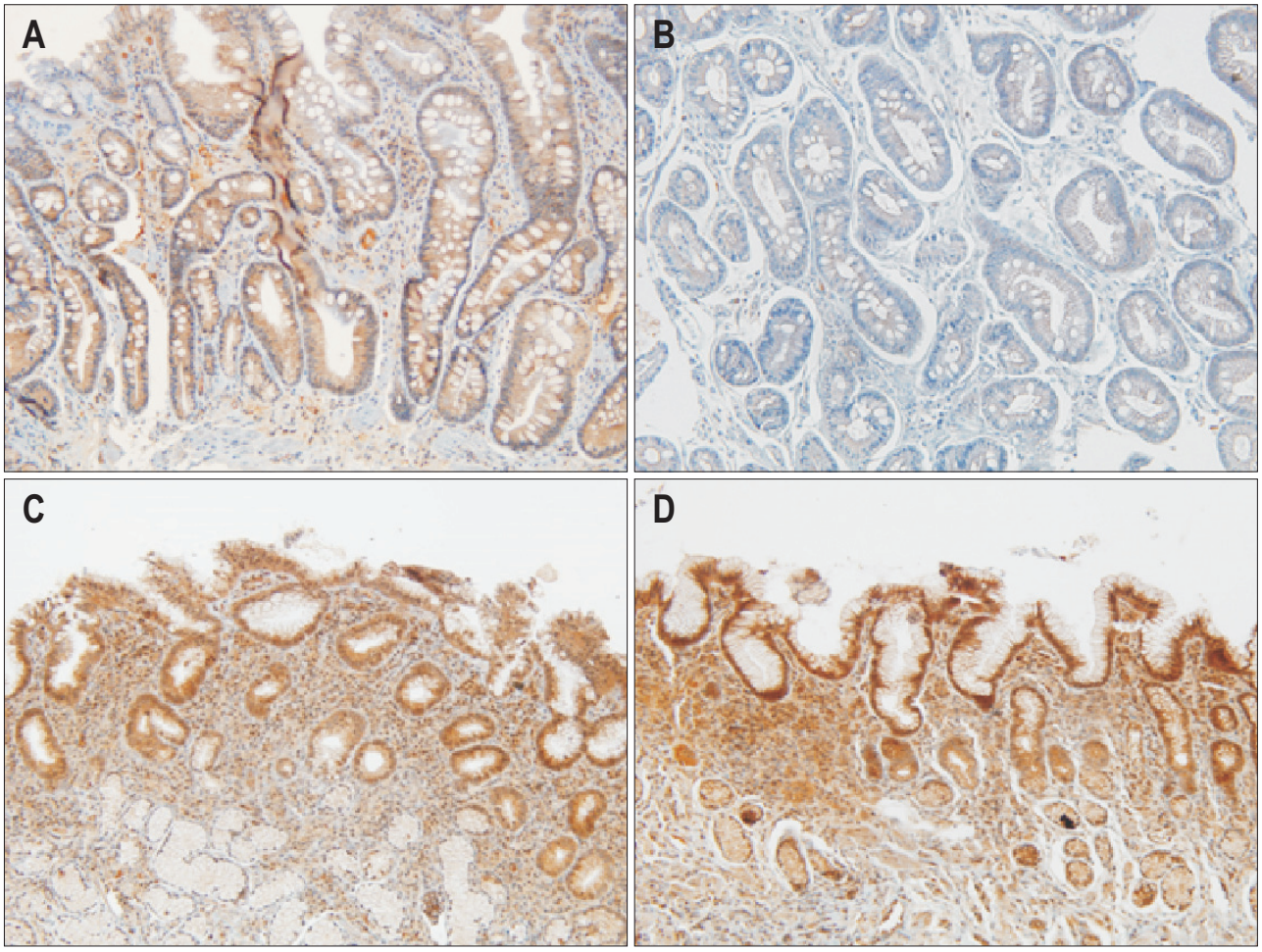


Fig. 4. Representative immunohistochemical staining for *cIAP2* protein in patients with *Helicobacter pylori*-positive early gastric cancer before and 12 months after endoscopic submucosal dissection (ESD) (original magnification, ×200). (A) Strong expression of cIAP2 in the eradication group before ESD; (B) faint expression of cIAP2 in the eradication group at the 12-month follow-up; (C) strong expression of cIAP2 in the noneradication group before ESD; (D) strong expression of cIAP2 in the noneradication group at the 12-month follow-up. In the eradication group, the expression of *cIAP2* protein decreased at 12-month follow-up. In the noneradication group, there was no difference in *cIAP2* protein expression before and 12 months after ESD.

gastric cancer by *H. pylori* eradication after ESD in EGC patients may be related to changes in the expression of genes controlling cell proliferation and apoptosis.

There are two distinct apoptotic pathways: a death receptor (extrinsic) pathway and a mitochondrial (intrinsic) pathway.[24] Some reports claim that overexpression of *cIAP2* inhibits apoptosis through the suppression of caspases 3, 7, and 9 activity.[25,26] Others suggest that the underlying mechanism involves the suppression of caspase 8 activity.[4] In this study, several caspase genes were probed with a real-time PCR microarray. We found that caspases 3, 8, and 9 were suppressed but caspase 7 was slightly overexpressed in subjects with *H. pylori*-positive EGC compared to that in *H. pylori*-negative individuals without AG/IM. These findings were somewhat counterintuitive. Moreover, *Bcl2* and *Bax*, which play opposing roles in the intrinsic pathway, were equally overexpressed in *H. pylori*-positive EGC patients. Because we examined the expression of general apoptosis-related genes in a very limited number of samples, and the interactions of the intrinsic and extrinsic apoptosis pathways are complex, we could not determine whether *Bax* was overexpressed to counteract the overexpression of *Bcl2*. Further experiments are required to expose the pathways key to *H. pylori*-infected and *cIAP2*-induced gastric carcinogenesis.

This study had several limitations. First, we did not determine the status of *CagA* in the *H. pylori* strains in our study; hence, it was not possible to ascertain whether only *CagA*-positive *H. pylori* induces *cIAP2* overexpression during gastric carcinogenesis. Moreover, lack of assessment of other virulent factors such as *VacA* and *OipA*, which are frequently found in Asian strains, is also a limitation.[27] Second, because there was a limit to the number of available tissues from noncancerous subjects, we could not evaluate the effects of AG and IM on the expression of *cIAP2* separately. Although evidence suggested that *cIAP2* was overexpressed at a relatively late stage during *H. pylori*-

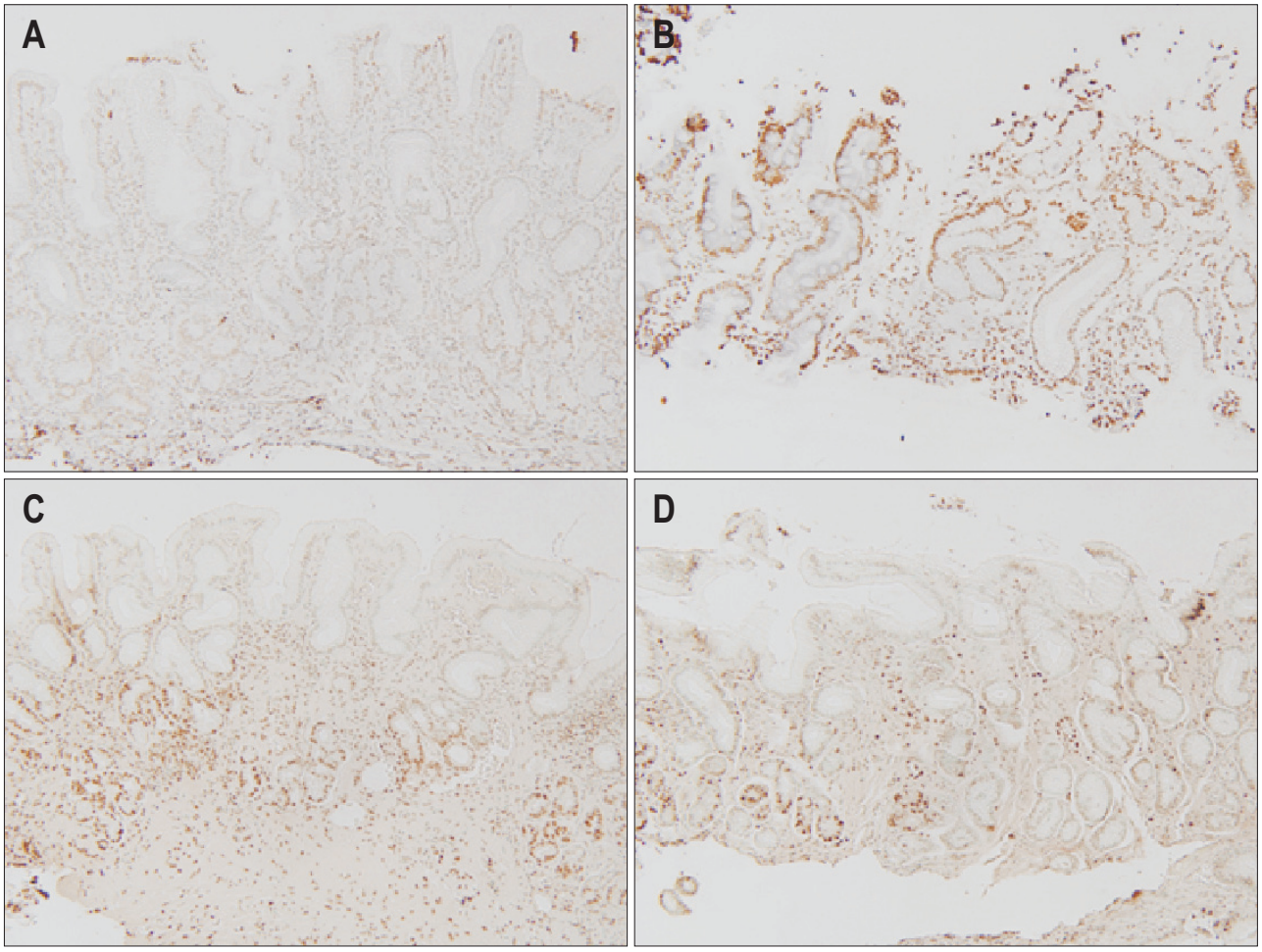


Fig. 5. Representative terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining of gastric tissues from patients with *Helicobacter pylori*-positive early gastric cancer before endoscopic submucosal dissection (ESD) and at the 12-month follow-up (original magnification, ×200). (A) Eradication group before ESD; (B) eradication group at the 12-month follow-up; (C) noneradication group before ESD; (D) noneradication group at the 12-month follow-up. In the eradication group, cell apoptosis assayed by using TUNEL increased at the 12-month follow-up. In the noneradication group, there was no difference in cell apoptosis before ESD and at the 12-month follow-up.

induced gastric carcinogenesis, the specific stage at which *cIAP2* is overexpressed remains unknown. Finally, we did not examine more potent anti-apoptotic factors such as *Bcl2*-family member *Mcl1*.[28] We also did not evaluate the status of tumor necrosis factor-related apoptosis-inducing ligand signaling and *cIAP2*-counteracting molecules such as *Smac/DIABLO* and *Omi/Htr2A*.[29] Therefore, it remains uncertain whether *H. pylori* eradication-mediated *cIAP2* downregulation alone induces apoptosis in the eradication group, as other signaling events may be involved.

In conclusion, the expression of *cIAP2* increased during gastric carcinogenesis after *H. pylori* infection and progression to AG/IM, and eradication of *H. pylori* in the patients who underwent ESD for EGC reversed the overexpression of *cIAP2* and suppressed cell apoptosis.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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