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## Expression of bcl-2 oncogene in gastric precancerous lesions and its correlation with syndromes in traditional Chinese medicine

Ling Hu, Shao-Xian Lao, Chun-Zhi Tang

Ling Hu, Shao-Xian Lao, Chun-Zhi Tang, Institute of the Spleen and Stomach, Guangzhou University of Traditional Chinese Medicine, Guangzhou 510405, Guangdong Province, China  
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Co-correspondents: Shao-Xian Lao

Correspondence to: Ling Hu, Institute of the Spleen and Stomach, Guangzhou University of Traditional Chinese Medicine, 12 Jichang Road, Guangzhou 510405, Guangdong Province, China. pqhl@yahoo.com.cn

Telephone: +86-20-36585444

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### Abstract

**AIM:** To observe the protein and mRNA expression of bcl-2 oncogene in gastric precancerous lesions (GPL) and to analyze its correlation with syndromes in traditional Chinese medicine (TCM).

**METHODS:** Sixty-seven patients with GPL confirmed by gastroscopy and pathology were studied, including 39 cases of moderate gastric mucosal dysplasia, 19 cases of severe gastric mucosa dysplasia, 9 cases of incomplete colon metaplasia. In syndrome differentiation of TCM, 17 cases belonged to the syndrome of qi and yin deficiency of the spleen and stomach complicated by qi stagnation, 21 cases belonged to the syndrome of qi and yin deficiency of the spleen and stomach complicated by stomach heat, 29 cases belonged to the syndrome of qi and yin deficiency of the spleen and stomach complicated by blood stasis. Protein and mRNA expression of bcl-2 oncogene were detected by labeled streptavidin biotin (LSAB) immunohistochemistry and *in situ* hybridization respectively.

**RESULTS:** Abnormal expression of protein and mRNA on bcl-2 oncogene was found in GPL, which increased gradually with the course of lesions. In moderate and severe gastric mucosal dysplasia and incomplete colon metaplasia, there was no difference in the expression of bcl-2 oncogene ( $P>0.05$ ). In different accompanying syndromes, the expression of protein and mRNA on bcl-2 oncogene increased gradually in the following order: deficiency of both qi and yin of the spleen and stomach accompanying qi stagnation → stomach heat → blood stasis. In GPL, compared with accompanying blood stasis, there was an obvious difference in the expression of bcl-2 oncogene between the syndrome of qi and yin deficiency of the spleen and stomach and accompanying stomach

heat, so did accompanying qi stagnation (the level of protein:  $\chi^2 = 8.45$ ,  $P<0.05$ ; the level of mRNA:  $\chi^2 = 7.35$ ,  $P<0.05$ ).

**CONCLUSION:** Apoptosis-associated bcl-2 oncogene is abnormally expressed in GPL, which correlates with different accompanying syndromes in TCM.

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**Key words:** Stomach neoplasm/genetics; Stomach neoplasm/therapy of TCM; Precancerous condition/pathology; Oncogene bcl-2; Syndrome of TCM

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### INTRODUCTION

Gastric precancerous lesions (GPL) refer to moderate and severe dysplasia and/or incomplete colon metaplasia. It has an increasing risk to develop into gastric cancer<sup>[1-5]</sup>. Gastric cancer has a close correlation with abnormal cell proliferation and apoptosis. Excessive accumulation of cells caused by imbalance of proliferation and apoptosis may be the pathological basis of gastric cancer development<sup>[6-10]</sup>. Traditional Chinese medicine (TCM) holds that GPL is a complex syndrome of deficient origin and excessive superficiality. Deficiency of qi and yin of the spleen and stomach is the origin of GPL. Its superficiality includes qi stagnation, blood stasis and heat toxin. Chinese herbs can not only relieve clinical symptoms of GPL<sup>[11-13]</sup>, but also regulate the expression of related oncogenes to some extent in GPL<sup>[14-17]</sup>. Bcl-2 is one of the oncogenes and has a close correlation with apoptosis, its abnormal expression is usually observed during the development of GPL<sup>[18-25]</sup>. Although overexpression of bcl-2 oncogene is found in GPL, it is different in same tissues of different sufferers and the regulating effect of Chinese herbs is different too. Recently, it has been found that the protein expression of some proliferation-associated genes is correlated with GPL syndromes<sup>[26]</sup>. In this article, the correlation between expression of bcl-2 oncogene in GPL with syndromes in TCM was further investigated from the viewpoints of apoptosis, protein expression and mRNA transcription of bcl-2 oncogene.

## MATERIALS AND METHODS

### Materials

All samples of gastric mucosa were obtained under a gastroscopy. Four samples were obtained from each patient. Moderate and severe dysplasia were confirmed by pathologic examination. Incomplete colon metaplasia was confirmed by HID-ABpH2.5-PAS staining. Based on the syndrome differentiation in TCM, patients meeting the diagnostic criteria were included in the study, patients with abnormal functions of heart, liver, and kidney or carcinomatous change were excluded. The criteria of syndrome differentiation in TCM were as follows (referring to Clinical Trial Guideline of New Chinese Herbs, first edition, 1993:88, 108); dominating symptoms of deficiency of both qi and yin of the spleen and stomach: including fullness and distension of the epigastrium being more severe after meal, bulgy and tender tongue, or tongue with tooth prints, weak red tongue with whitish fur or thin fur, slow and weak pulse; minor symptoms of deficiency of both qi and yin of the spleen and stomach: including lassitude of limbs, no desire to speak, sweating, dryness of mouth and without desire to drink, emaciation. Patients with two dominating symptoms (including state of tongue or pulse) and two minor symptoms could be diagnosed as dysplasia. Accompanying symptoms of qi stagnation were as follows: fullness, distension and discomfort of the epigastrium, distending pain in hypochondrium, frequent belching, gastric discomfort with acid regurgitation and taut pulse. Accompanying symptoms of blood stasis were as follows: persistent and fixed pain, sting, dark tongue or tongue with petechia. Accompanying symptoms of stomach heat were as follows: dry and bitter mouth, dry feces, and tongue with yellow fur. Patients having two of the above symptoms were diagnosed with accompanying syndromes. Sixty-seven cases of GPL were enrolled in this research, including 39 cases of moderate gastric mucosal dysplasia, 19 cases of severe gastric mucosa dysplasia and 9 cases of incomplete colon metaplasia. In the syndrome differentiation in TCM, 17 cases had qi stagnation, 21 cases had stomach heat, and 29 cases had blood stasis. Their average age was  $53 \pm 11$  years. The course of disease was less than 5 years in 36 cases, 5-10 years in 25 cases, and more than 10 years in 6 cases. Monoclonal antibody to bcl-2 and LSAB reagent kit were products of DAKO Company. Bcl-2 ISH kit was a product of Boshide Company.

### Methods

Gastric mucosa was prepared with 1 g/L diethyl pyrocarbonate before it was fixed for preventing degeneration of nuclear acids. Labeled streptavidin biotin method and *in situ* hybridization (ISH) were adopted for determining the expression of protein and mRNA transcription of bcl-2 oncogene. PBS was used instead of the first antibody as negative control group. Proved positive sections were used as positive control. Positive signals were fine brown yellow granules. The hybridization probe was removed in negative group. The samples were prepared with RNase for preventing false positive signals. The positive signals were fine purple and black granules.

### Statistical analysis

The data were analyzed by  $\chi^2$  test and  $\chi^2$  partition test with SPSS 10.0 software package.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Detection of bcl-2 oncogene in GPL

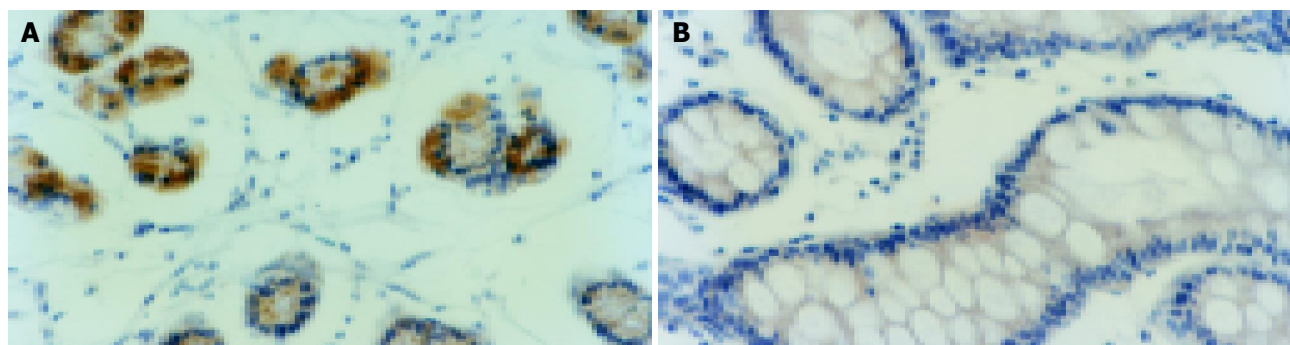
The expression of protein and mRNA on bcl-2 oncogene was observed in cytoplasm of GPL (Figures 1 and 2). As the lesion developed, the expression gradually increased, but there was no significant difference in moderate and severe gastric mucosal dysplasia and incomplete colon metaplasia ( $P > 0.05$ , Table 1).

**Table 1** Protein and mRNA expression of bcl-2 oncogene in GPL (%)

| Lesions                            | n  | Protein expression | mRNA expression |
|------------------------------------|----|--------------------|-----------------|
| Moderate gastric mucosal dysplasia | 39 | 35.89              | 41.03           |
| Severe gastric mucosal dysplasia   | 19 | 47.37              | 52.63           |
| Incomplete colon metaplasia        | 9  | 33.33              | 44.44           |

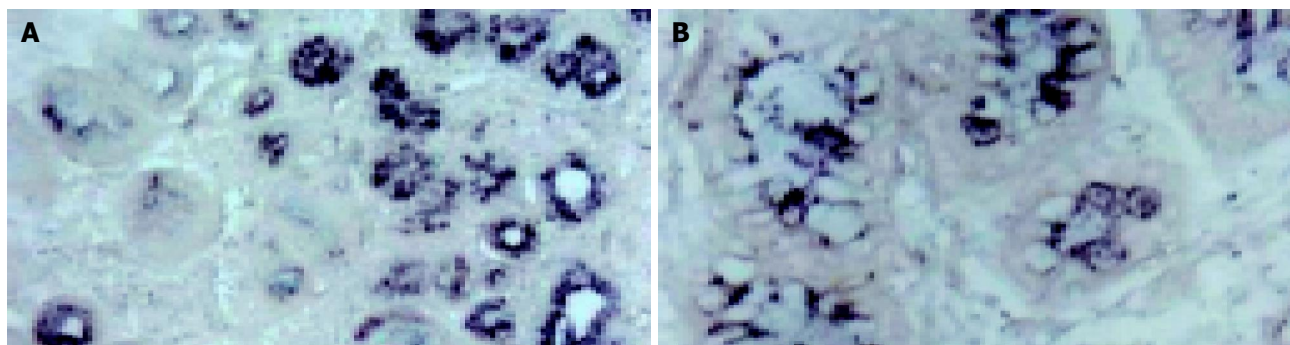
### Expression of bcl-2 oncogene in different accompanying syndromes

The protein and mRNA expressions of bcl-2 oncogene were observed in different accompanying syndromes. The expression increased gradually in the following order: deficiency of both qi and yin of the spleen and stomach accompanying qi stagnation → stomach heat → blood stasis (Table 2).



**Figure 1** Protein positive expression of bcl-2 oncogene in gastric mucosal

dysplasia. (A) and incomplete colon metaplasia; (B) (LSAB,  $\times 200$ ).



**Figure 2** mRNA positive expression of bcl-2 oncogene in gastric mucosal

dysplasia. (A) and incomplete colon metaplasia; (B) (ISH,  $\times 200$ ).

**Table 2** Protein and mRNA expression of bcl-2 oncogene in GPL with different accompanying syndromes (%)

| Accompanying syndrome | n  | Protein expression | mRNA expression    |
|-----------------------|----|--------------------|--------------------|
| Qi stagnation         | 17 | 17.65              | 23.53              |
| Stomach heat          | 21 | 28.57              | 33.33              |
| Blood stasis          | 29 | 58.62 <sup>a</sup> | 62.07 <sup>c</sup> |

<sup>a</sup> $P < 0.05$ ,  $\chi^2$ : 8.45 *vs* stagnation and stomach heat. <sup>c</sup> $P < 0.05$ ,  $\chi^2$ : 7.35 *vs* qi stagnation and stomach heat.

## DISCUSSION

Oncogene can regulate and control proliferation, differentiation metastasis and apoptosis of carcinoma cells<sup>[27,28]</sup>. Bcl-2 is a new oncogene, which locates on 18q21 of chromosome, encodes a protein with *M*<sub>r</sub> 26 000 molecule, and is predominantly distributed in nuclear membrane and cytoplasm of endoplasmic reticulum. It can block programmed cell death but does not affect cell proliferation and is an important factor for suppressing apoptosis<sup>[29]</sup>. Overexpression of bcl-2 oncogene promotes development of carcinoma by suppressing apoptosis and prolonging life span of cells<sup>[30,31]</sup>. Under normal conditions, cells of human gastric mucosa renew frequently. The dynamic balance between proliferation and apoptosis maintains the normal structure of gastric mucosa. Generally speaking, apoptotic cells locate on the surface of mucosa and proliferate on the gland neck of gastric mucosa, indicating the growing, aging and dying rule of gastric mucosa cells from basal layer to surface. However, in atrophy gastritis, colon metaplasia, dysplasia and gastric cancer, apoptotic and proliferating cells do not have the above characteristics, and abnormal expression of bcl-2 oncogene is found in these lesions. When the lesions exacerbate, the cell proliferation index increases while apoptosis index decreases gradually, the dynamic balance between proliferation and apoptosis is disturbed, suggesting that there is a regulating disturbance between cell apoptosis and proliferation in carcinogenesis of gastric mucosa, while bcl-2 oncogene is involved in regulating apoptosis of GPL<sup>[6-10]</sup>. Finally, the increase of instability and speeding of accumulation of cells result in carcinogenesis.

TCM believes that GPL is a syndrome of deficient origin and excessive superficiality. Deficiency of both qi and yin of the spleen and stomach is the origin of GPL. Its superficiality includes qi stagnation, blood stasis, and heat toxin. Chinese

herbs can not only relieve the clinical symptoms of GPL<sup>[11-13]</sup>, but also decrease the protein expression of bcl-2 oncogene to some extent<sup>[32,33]</sup>. The expression of bcl-2 oncogene is different in similar tissues of different patients, while the regulating effect of Chinese herbs on gene expression is not the same. GPL is a complex syndrome of deficient origin and excessive superficiality. Does the difference indicate that there is an internal relationship between the expression of apoptosis-associated gene and syndromes in TCM? The results of our study showed that there were abnormal protein and mRNA expressions of bcl-2 oncogene in GPL. With the development of lesions in gastric mucosa, the positive rate of the gene expression increased gradually. Although there were no significant differences in expression of bcl-2 oncogene in moderate and severe gastric mucosal dysplasia and incomplete colon metaplasia, it was quite different between different accompanying syndromes in TCM. From the viewpoint of TCM, the state of illness is the slightest in accompanying qi stagnation, more severe in accompanying stomach heat, and the worst in accompanying blood stasis, indicating that there is a parallel relationship between the expression of bcl-2 oncogene and the state of illness in different accompanying syndromes of GPL. The more serious the state of illness, the higher is the positive rate of bcl-2 oncogene expression. The pathology of transformation of accompanying syndromes of GPL correlates with the abnormal expression of proliferation-associated genes<sup>[26]</sup> and apoptosis-associated genes, which is consistent with the concept that carcinoma results from the imbalance between cell proliferation and apoptosis in Western medicine. Study on the correlation between the syndromes of GPL and gene expression might be a new way for uncovering the syndromes of GPL.

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