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*Original Article*

# Detecting of gastric cancer by Bcl-2 and Ki67

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**Abstract:** Gastric cancer is one of the most common malignant tumors and the second leading cause of cancer-related deaths in China. Although there is some progress in diagnose and treatment, the incidence of gastric cancer still keeps up increasing. In this study 40 patients with gastric cancer who underwent surgical operation is detected by immunohistochemistry. The positive rates of Bcl-2 and Ki67 protein expression in gastric cancer tissues were significantly higher than normal gastric mucous tissues. Correlation analysis showed that the expression of Bcl-2 is not correlated with that of Ki67. Positive expression of Bcl-2 or Ki67 did not correlate with age, gender, differentiation, stage and lymph node metastasis. These suggested that combination of Bcl-2 and Ki67 to detect gastric cancer is more effective.

**Keywords:** Bcl-2, Ki67, gastric cancer, immunohistochemistry

## Introduction

Gastric cancer is one of the most common malignant tumors and causes approximately 800,000 deaths worldwide per year [1]. In China, the morbidity of gastric cancer has the first place among malignant tumors. Gastric cancer is the second leading cause of cancer-related deaths in China [2]. In recent years the incidence of gastric cancer still keeps up increasing, although there is some progress in diagnose and treatment [3]. The identification of prognostic factors in gastric cancer was essential for diagnosing patients and determining optimal therapeutic strategies.

## Materials and methods

### *Patients*

40 patients with gastric cancer who underwent surgical operation in the First Affiliated Hospital, Xi'an Medical University, 28 males and 12 females, were enrolled in the study. The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from all participants.

### *Immunohistochemistry*

The tissue specimens were formalin (10%) fixed and paraffin-embedded using standard technique. Each paraffin embedded tissue specimen was microtomed into four serial sections, of 4 μm thickness each. Two sections were used for H&E staining, one for Bcl-2 staining, and the other for Ki67 staining. Anti- Bcl-2 and -Ki67 monoclonal antibodies, and DAB reagent were purchased from Beijing Zhongshan Inc. China. The sections were stained with hematoxylin and DAB according to the manufacturer's instructions.

### *Statistical analysis*

Quantitative variables were compared using the Pearson's chi-square test or Pearson's relation probability test by analysis of variance. The statistical significance was accepted for $P<0.05$. All analyses were performed using the SPSS version 16.0 (SPSS, Chicago, IL).

## Results

### *The expressions of Bcl-2 and Ki67 in gastric cancer*

The cancer and normal tissues of 40 patients with gastric cancer were detected by immunohistochemistry. Immunochemical staining tests showed that Bcl-2 was mainly localized in the cytoplasm and Ki67 in nuclear of gastric cancer cells (**Figure 1**). The positive rates of Bcl-2 and

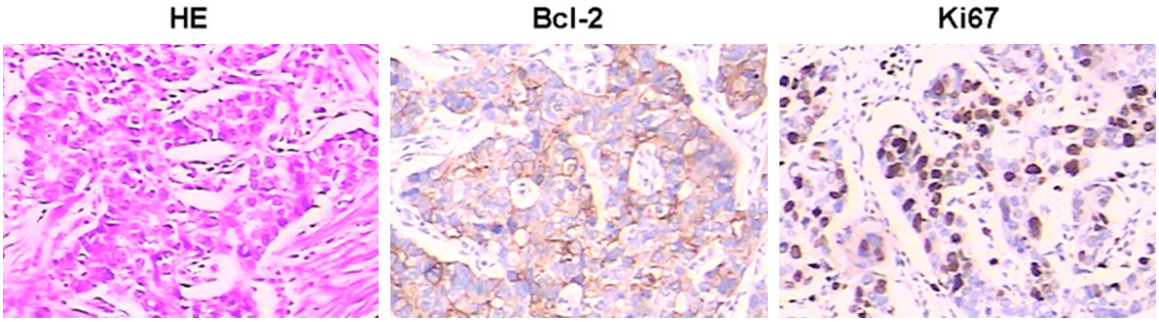


**Figure 1.** The expressions of Bcl-2 and Ki67 in gastric cancer were detected by immunohistochemistry (200×).

**Table 1.** The correlation analysis of expression of Bcl-2 and Ki67

| | Bcl-2 | | r | P |
|---|---|---|---|---|
| | Positive | Negative | | |
| Ki67 | | | | |
| Positive | 18 | 10 | 1.415 | 0.234 |
| Negative | 10 | 2 | | |

Ki67 protein expression in gastric cancer tissues were both 70%. However, nothing of normal gastric mucous tissues express the protein of Ki67 and only 20% of normal tissues expressed Bcl-2, which differed significantly ($P<0.05$) from the corresponding positive rates in the cancer tissues.

Correlation analysis showed that the expression of Bcl-2 is not correlated with that of Ki67 ($P=0.234$, **Table 1**). The total negative rate of Bcl-2 and Ki67 is only 5% (2/40), which reduce efficiently the missing diagnose of gastric cancer.

*Overexpression of f Bcl-2 or Ki67 and clinic pathological factors*

The relationship between expression of Bcl-2 or Ki67 and clinic pathological factors was investigated in gastric cancer tissues. Positive expression of Bcl-2 did not correlate with age, gender, differentiation, stage and lymph node metastasis (P>0.05, **Table 2**). In addition, Ki67expression did not correlate with the clinic pathological factors either (P>0.05, **Table 3**).

## Discussion

Bcl-2 is an anti-apoptotic protein, which localized in intracellular membranes, mostly in the outer mitochondrial membrane, nuclear membrane, and the endoplasmic reticulum [4]. Bcl-2 regulates ion channels, caspase status, and cytochrome c localization, and has an anti-apoptotic function [5].

Ki-67 is an antigen associated with cell proliferation [6]. Ki-67 is expressed during the proliferation and synthesis phases of the cell cycle (G1, S, G2, and M), and however it is not expressed during G0 phase [7].

Bcl-2 has been reported in a variety of human epithelial malignant tumors including gastric carcinoma [8]. Bcl-2 protein was not detected in chronic gastritis, but aberrant expression was found in gastric epithelial intestinal metaplasia and dysplasia. The overexpression of Bcl-2 protein is an early event in gastric tumorigenesis, before gastric dysplastic changes occur [9]. The expression of bcl-2 was an independent prognostic factor for patients with gastric cancer; it might be a candidate for the gastric cancer staging system [10]. Bcl-2 expression has an additional contribution in predicting response to this chemotherapy combination [11, 12]. However, Martin-Arruti et al reported that Bcl-2 expression does correlate with worse prognosis [13].

The detection of expression of Ki67 in gastric cancer may provide useful prognostic information for patients with the disease [14-16]. The overexpression of Ki67 was negatively correlated with carcinoma differentiation [17]. The routine evaluation of Ki67 levels could be a useful tool in identification of patient with more aggressive disease and contribute to a better therapeutic approach [18]. A study showed that Bcl-2 and Ki-67 expression and apoptosis were not different among patients with and without a history of gastric cancer in first degree rela-

**Table 2.** Relationship between expression of Bcl-2 and clinicopathological parameters in gastric cancer

| Variable | Cases | Positive (%) | Negative (%) | $\chi^2$ | *P* value |
|---|---|---|---|---|---|
| Gender | | | | | |
| Male | 28 | 20 (71%) | 8 (29%) | 0.006 | 0.940 |
| Female | 12 | 8 (67%) | 4 (33%) | | |
| Age | | | | | |
| ≤60 | 18 | 12 (67%) | 6 (33%) | 0.173 | 0.677 |
| >60 | 22 | 16 (73%) | 6 (27%) | | |
| Differentiation | | | | | |
| High | 12 | 10 (83%) | 2 (17%) | | |
| Median | 14 | 10 (71%) | 4 (29%) | | |
| Low | 14 | 8 (57%) | 6 (43%) | 1.033 | 0.309 |
| Stage | | | | | |
| I/II | 12 | 8 (67%) | 4 (33%) | 0.006 | 0.940 |
| III/IV | 28 | 20 (71%) | 8 (29%) | | |
| LN metastasis | | | | | |
| Positive | 26 | 16 (62%) | 10 (62%) | 1.512 | 0.219 |
| Negative | 14 | 12 (86%) | 2 (14%) | | |

**Table 3.** Relationship between expression of Ki67 and clinicopathological parameters in gastric cancer

| Variable | Cases | Positive (%) | Negative (%) | $\chi^2$ | *P* value |
|---|---|---|---|---|---|
| Gender | | | | | |
| Male | 28 | 18 (64%) | 10 (36%) | 0.686 | 0.408 |
| Female | 12 | 10 (83%) | 2 (17%) | | |
| Age | | | | | |
| ≤60 | 18 | 12 (95%) | 6 (5%) | 0.005 | 0.945 |
| >60 | 22 | 16 (95%) | 6 (5%) | | |
| Differentiation | | | | | |
| High | 12 | 8(67%) | 4 (33%) | | |
| Median | 14 | 10 (71%) | 4 (29%) | | |
| Low | 14 | 10 (71%) | 4 (29%) | 0.027 | 0.870 |
| Stage | | | | | |
| I/II | 12 | 6 | 6 | 1.695 | 0.193 |
| III/IV | 28 | 20 | 8 | | |
| LN metastasis | | | | | |
| Positive | 26 | 18 | 8 | 0.584 | 0.445 |
| Negative | 14 | 8 | 6 | | |

tives8. Tsamandas et al reported that Bcl-2 expression did not correlate with Ki-67 [19].

In this study, we assessed the expression of Bcl-2 or Ki67 in gastric cancer tissues. Our data showed that the positive rates of Bcl-2 and Ki67 protein expression in gastric cancer tissues were higher than normal gastric mucous tissues. Correlation analysis showed that the expression of Bcl-2 is not correlated with that of Ki67. Positive expression of Bcl-2 or Ki67 did not correlate with age, gender, differentiation, stage and lymph node metastasis. The total negative rate of Bcl-2 and Ki67 is only 5%, which reduce efficiently the missing diagnose of gastric cancer.

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## Disclosure of conflict of interest

None.

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