

# Expression and Significance of Beclin-1 and Bcl-2 in Gastric Cancer

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**Abstract: Objective** To investigate the role and correlation of Beclin-1 and Bcl-2 in the occurrence and progression of gastric cancer. **Methods** The expression of Beclin-1 and Bcl-2 in 78 cases of gastric cancer, 30 cases of atypical hyperplasia and 20 cases of chronic non-atrophic gastritis was examined by immunohistochemical staining. **Results** The positive rate of Beclin-1 in gastric cancer was significantly lower than those in atypical hyperplasia and chronic non-atrophic gastritis ( $\chi^2=9.298, 7.437, P<0.05$ ), while there was no significant difference between atypical hyperplasia and chronic non-atrophic gastritis ( $P>0.05$ ). The positive rate of Bcl-2 in gastric cancer was significantly higher than those in atypical hyperplasia and chronic non-atrophic gastritis ( $\chi^2=11.013, 15.278, P<0.05$ ), while Bcl-2 expression in atypical hyperplasia showed no significant difference with that in chronic non-atrophic gastritis ( $P>0.05$ ). The expression of Beclin-1 and Bcl-2 was significantly correlated with differentiation, depth of invasion and TNM staging ( $\chi^2=7.014\sim 16.248, P>0.05$ ), but not correlated with sex, age, tumor size, tumor location and metastasis ( $P>0.05$ ). The expression of Beclin-1 was negatively correlated with Bcl-2 in gastric cancer ( $r=-0.396, P<0.01$ ). **Conclusion** Beclin-1 expression was decreased and Bcl-2 expression was increased in gastric cancer. Both were associated with the occurrence and progression of gastric cancer and they were negatively correlated. Beclin-1 and Bcl-2 may be used as important markers to predict the biological behavior of gastric cancer.

**Keywords:** gastric cancer, Beclin-1, Bcl-2, autophagy, apoptosis

## Introduction

Gastric cancer is one of the most common malignancy. Studying the mechanism of its occurrence and progression is of great clinical significance for early prevention and treatment of the disease. Tumorigenesis is associated with the dynamic imbalance between cell proliferation and cell death. Autophagy and apoptosis regulate proliferation and consumption of tumor cells, and are closely related to the occurrence and progression of tumors[1]. Beclin-1, one of the key autophagy-associated proteins, plays an crucial role in regulating the process of autophagy and has been recognized as an important marker to assess the level of autophagy[2]. Anti-apoptosis is one of the important characteristics of tumor cells. The Bcl-2 anti-apoptotic protein is one of the important members of the apoptosis-associated Bcl-2 family proteins. It can inhibit apoptosis and increase the chances of chromosome aberration and virus infection, and therefore leading to cell malignant transformation and tumorigenesis[3]. To the best of our knowledge, the correlation of Beclin-1 and Bcl-2 in gastric cancer has not been reported yet. This study aimed to detect the expression of Beclin-1 and Bcl-2 in gastric cancer,

and investigate the correlation between them and their associations with clinical pathological parameters of patients.

## Materials and Methods

Formalin-fixed, paraffin-embedded 78 gastric cancer specimens, 30 atypical hyperplasia specimens and 20 chronic non-atrophic gastritis specimens were respectively obtained from the patients who diagnosed with the corresponding diseases in the Affiliated Hospital of Qingdao University between 2013 and 2015. None of these patients had ever received any preoperative treatment, such as radiation and chemotherapy.

## Immunohistochemical staining and assessment

Beclin-1 was detected with a rabbit polyclonal antibody (1:200, Boster-bio, Wuhan, China) and Bcl-2 was detected with rabbit monoclonal antibody (1:100, Maxin-bio, Fuzhou, China). Formalin-fixed, paraffin-embedded sections (3 $\mu$ m thick) were cut from the paraffin blocks and mounted on slides coated with 3-aminopropyl-triethoxysilane. Briefly, after deparaffinization and rehydration, heat-induced epitope retrieval was conducted and tissues were

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incubated with primary antibodies for 60 min at room temperature, followed by incubation for 30 min with the secondary antibody(Maxin-bio, Fuzhou, China). Breast cancer and amygdaline tissue were used as positive controls for Beclin-1 and Bcl-2 respectively. PBS was used as a negative control in place of primary antibody.

The slides were reviewed independently by two pathologists blinded to clinical details. The expression of Beclin-1 and Bcl-2 was evaluated using a scoring system based on staining intensity and percentage of positive carcinoma cells. The staining intensity was classified into four categories: 0,negative; 1,weak; 2,moderate; 3,strong. The percentage of positive tumor cells was scored as 0(<5%),1(6%to25%),2(26%to50%),3(51%to70%) and 4(>70%). The score of each slide was calculated by multiplying the score of staining intensity by the score of positive rate. The final score 3 was used as the cutoff to separate tumors into high expression or low expression group.

**Statistics**

All analyses were performed using SPSS 17.0 software. The positive rates among all group were compared with the Chi-square test. Pearson correlation test was used to analyze the correlation of Beclin-1 and Bcl-2.

**Results**

Expression of Beclin-1 and its correlations with clinicopathological parameters

Beclin-1 was positively localized in the cytoplasm(Fig.1). The positive rate of Beclin-1 in gastric cancer was significantly lower than those in atypical hyperplasia and chronic non-atrophic gastritis( $\chi^2=9.298,7.437, P<0.05$ ), while there was no significant difference between atypical hyperplasia and chronic non-atrophic gastritis ( $P>0.05$ ). (Fig. 1, Table 1 ).Beclin-1 expression in gastric cancer was significantly correlated with differentiation, depth of invasion and TNM staging, but not correlated with sex, age, tumor size, tumor location and metastasis(Table 2).

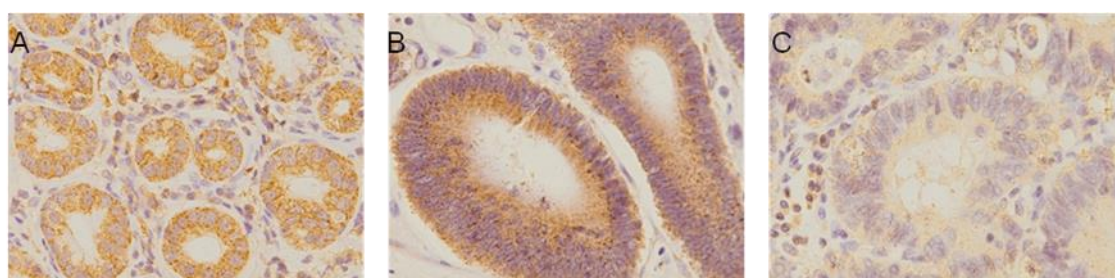


Fig.1 Beclin-1 expression in chronic non-atrophic gastritis (A), atypical hyperplasia (B) and gastric cancer(C)(SP,400)

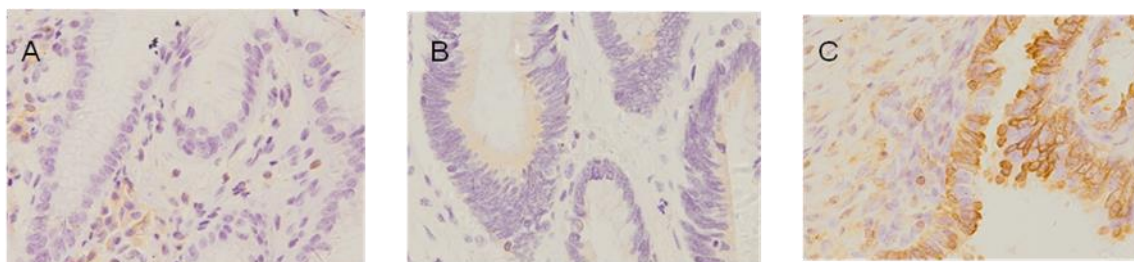


Fig.2 Bcl-2 expression in chronic non-atrophic gastritis (A), atypical hyperplasia (B) and gastric cancer(C)(SP,400)

Table 1 The positive rate of Beclin-1 and Bcl-2 in non-atrophic gastritis ,atypical hyperplasia and gastric cancer ( n ( % ) )

Groups	n	Beclin-1	Bcl-2
Control group	20	17 ( 85.0 )	2 ( 10.0 )
Atypical hyperplasia	30	25 ( 83.3 )	7 ( 23.3 )
Gastric cancer	78	40 ( 51.3 )	46 ( 59.0 )

Expression of Bcl-2 and its correlations with clinicopathological parameters

Bcl-2 was positively localized in cell membrane and cytoplasm(Fig.2). The positive rate of Bcl-2 in gastric cancer was significantly higher than those in atypical hyperplasia and chronic non-atrophic gastritis( $\chi^2=11.013, 15.278, P<0.05$ ), while Bcl-2 expression in atypical hyperplasia showed no

significant difference with that in chronic non-atrophic gastritis ( $P>0.05$ ). (Fig.2, Table 1 ).Bcl-2 expression in gastric cancer was significantly correlated with differentiation, depth of invasion and TNM staging, but not correlated with sex, age, tumor size, tumor location and metastasis(Table 2).

Table 2 Beclin-1 and Bcl-2 expression and clinicopathological parameters

clinicopathological parameters	N	Beclin-1			Bcl-2		
		+	$\chi^2$	P	+	$\chi^2$	P
Sex							
Male	56	32	2.730	>0.05	33	0.000	>0.05
Female	22	8			13		
Age							
$\geq 60$	47	25	0.173	>0.05	27	0.114	>0.05
<60	31	15			19		
Tumor size							
>5cm	21	9	1.565	>0.05	16	3.316	>0.05
$\leq 5$ cm	42	25			22		
Location							
Cardia-fundus	10	6	1.307	>0.05	4	2.564	>0.05
Corpus	11	4			8		
Antrum	38	20			24		
Differentiation							
Well	16	14	10.586	<0.05	5	7.014	<0.05
Moderate	28	12			17		
Poor	34	14			24		
Metastasis							
No	30	20	4.707	>0.05	15	4.154	>0.05
Lymph node metastasis	30	13			22		
Distant metastasis	18	7			9		
Depth of invasion							
T <sub>1</sub>	17	13	10.875	<0.05	5	14.582	<0.05
T <sub>2</sub>	8	7			3		
T <sub>3</sub>	39	15			31		
TNM staging							
I	20	18	16.248	<0.05	6	13.316	<0.05
II	20	7			15		
III	20	8			16		
IV	18	7			9		

Correlation of Beclin-1 and Bcl-2 in gastric cancer

Correlation analysis showed that the expression of Beclin-1 was negatively correlated with Bcl-2( $r=-0.396, P<0.01$ ).

**Discussion**

Autophagy is a complex process responsible for degradation of long-lived proteins or damaged organelles by lysosomes and recycling the product. Beclin-1 gene is a homologous gene of yeast ATG-6 (autophagy associated gene-6). Beclin-1 protein regulates the early process of the autophagic vacuole formation and plays an important role in recruiting other autophagy-associated proteins to form the preautophagosomal structure. Beclin-1 directly involves in the formation of the mammalian autophagosome and regulates autophagy activity[4].

This study showed that Beclin-1 expression in gastric cancer was significantly lower than those in atypical hyperplasia and chronic non-atrophic gastritis. This indicated that decreased Beclin-1 expression was associated with the occurrence of gastric cancer. This is in accordance with the previous studies in breast cancer[5], esophageal squamous cell carcinoma[6] and non-small cell lung cancer[7]. In this study, we found that Beclin-1 expression was significantly correlated with differentiation, depth of invasion and TNM staging. We also demonstrated that Beclin-1 expression of stage I gastric cancer was significantly

higher than those of stage II, III, IV. These results indicated that decreased Beclin-1 expression was correlated to the progression of gastric cancer, especially in the progression of stage I gastric cancer. These results suggest that Beclin-1 can be used as one of the important markers to predict biological behavior of gastric cancer.

The Bcl-2 (B-cell lymphoma/leukemia-2) gene is also called B lymphocyte tumor/leukemia-2 gene. It resides on chromosome 18q21. Bcl-2 protein can form Bax-Bcl-2 heterologous dimer with proapoptotic protein Bax of Bcl-2 family proteins, and therefore inhibiting cell apoptosis, prolonging the cell survival and increasing chances of malignant transformation[8]. Bcl-2 involves in the occurrence and progression of many tumors, such as pancreatic cancer[9], colorectal cancer[10]. Our study showed that the positive rate of Bcl-2 expression was significantly higher in gastric cancer group compared with the control groups. This indicated that Bcl-2 may be associated with the occurrence of gastric cancer. In addition, we found that Bcl-2 expression in gastric cancer was correlated with differentiation, TNM staging and depth of invasion. These results suggested that Bcl-2 played an important role in the progression of gastric cancer.

Our study showed that Beclin-1 expression was decreased and Bcl-2 expression was increased in gastric cancer, and they were negatively correlated. These results suggested that decreased autophagy and increased antiapoptosis were closely related in the occurrence and progression of gastric cancer. This is in accordance with the study of pancreatic cancer[3]. Kotfasti, et al.[11]found that the Bcl-2 can bind to BH3 domain of Beclin-1, forming Beclin-1/Bcl-2 compounds, and therefore lead to the inhibition of autophagy. Du, et al.[12] transferred the Bcl-2 specific siRNA to SGC7901 gastric cancer cells with high Bcl-2 expression. They found that Beclin-1 expression of the cells was significantly increased in both mRNA and protein levels. These results suggested that Bcl-2 could inhibit Beclin-1-dependent autophagy. In this study, the negative correlation of Bcl-2 and Beclin-1 expression may due to the Bcl-2 autophagy of gastric cancer cells.

In conclusion, Beclin-1 expression was decreased and Bcl-2 expression was increased in gastric cancer. They were negatively correlated and their expression in gastric cancer was significantly correlated with differentiation, depth of invasion and TNM staging. These results suggested that the decreased autophagy and the increased apoptosis were closely associated with the occurrence and progression of gastric cancer. Beclin-1 and Bcl-2 may be used as important markers to predict the biological behavior of gastric cancer.

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