

# Expression of Survivin in pancreatic cancer and its correlation to expression of Bcl-2

Jian-Guo Qiao, Yu-Qing Zhang, Yu-Chun Yin, Zui Tan

**Jian-Guo Qiao, Yu-Chun Yin, Zui Tan**, Department of General Surgery, Zhongnan Hospital Affiliated to Wuhan University, Wuhan 430071, Hubei Province, China

**Yu-Qing Zhang**, Medical Department of Wuhan University, Wuhan 430071, Hubei Province, China

**Correspondence to:** Zui Tan, Department of General Surgery, Zhongnan Hospital Affiliated to Wuhan University, Wuhan 430071, Hubei Province, China. txh728@public.wh.hb.cn

**Telephone:** +86-27-87325822

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## Abstract

**AIM:** To investigate the expression of Survivin in pancreatic cancer and its correlation to the expression of Bcl-2.

**METHODS:** Survivin and Bcl-2 expressions were examined by immunohistochemistry in 42 tissue samples from pancreatic cancer and 10 from normal pancreas.

**RESULTS:** No survivin expression was detected in the tissue samples from normal pancreas, while it was detected in 34 of 42 tissue samples from pancreatic cancer (81.95%). There was a correlation between survivin expression and differentiation and stages of pancreatic cancer. Survivin positive cases were strongly correlated to Bcl-2 expression (28/30 vs 6/12,  $P < 0.05$ ).

**CONCLUSION:** Overexpression of survivin plays an important role in the development and progression of pancreatic cancer, and correlates to the expression of Bcl-2. Survivin expression can be used as a prognostic factor.

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## INTRODUCTION

The inhibitor of apoptosis protein (IAP) is a member of the widely-expressed gene family of apoptosis inhibitors. It can inhibit apoptosis induced by a variety of stimuli and plays a critical role in the physiologic activities of cells. Survivin is a recently-characterized gene, a member of the IAP family, and has a close relation between anti-apoptosis and tumors. Overexpression of survivin has been reported to play an important role in the development and progression of pancreatic cancer<sup>[1-5]</sup>. In this study, we investigated the relationship between the expression of survivin in patients with pancreatic cancer and the expression of Bcl-2 in order to provide a theoretical basis for the prevention, diagnosis, and treatment of pancreatic cancer.

## MATERIALS AND METHODS

### Materials

Ten samples from normal pancreatic tissue and 42 samples from

pancreatic cancer were collected. Normal pancreatic tissues were obtained from autopsy specimens in Department of Anatomy, Medical College, Wuhan University, and their mean age was 49.3 years (range, 33-78). Pancreatic cancer samples were obtained from surgically-resected specimens in Zhongnan Hospital, Wuhan University. All these cancers were diagnosed pathologically.

### Reagents

Primary antibody for survivin was purchased from Novus Co., Ltd (USA) and Bcl-2 was purchased from Boster Biological Technology Ltd (Wuhan). SP kit was purchased from Zhongshan Biotechnology Co., Ltd (Beijing).

### Methods

Fixed in routinely-processed formalin and then embedded in paraffin, 4- $\mu$ m thick sections were prepared from the cut surface of blocks at the maximum cross-section. For morphological analysis, the sections were routinely stained with hematoxylin and eosin. Immunohistochemical staining for survivin and Bcl-2 antigen was made by the standard streptavidin/peroxidase (SP) technique. The positive section was used as a positive control. As a negative control, PBS was used instead of the primary antibody for survivin and Bcl-2.

### Scoring criteria

Cytoplasm staining with light yellow or brown was defined as a marker of positive cells. All sections were analyzed by an image analysis system. The mean percentage of positive cells for the expression of survivin and Bcl-2 was determined in at least 10 areas at 400-fold magnification, and the cases with less than 5% positively-stained cells were defined as being negative. The cases with 5% to 10% positively-stained cells were defined as having a positivity rate of "+", 11% to 60% as "++", and more than 60% as "+++".

### Statistical analysis

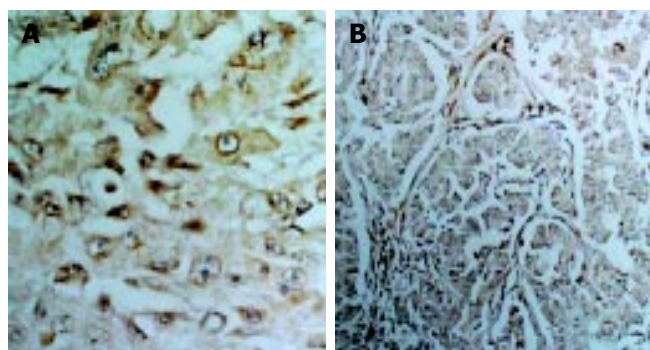
All statistical analyses were performed with SPSS 11.0 software. Difference and correlation were analyzed by  $\chi^2$  test.  $P < 0.05$  was considered statistically significant.

## RESULTS

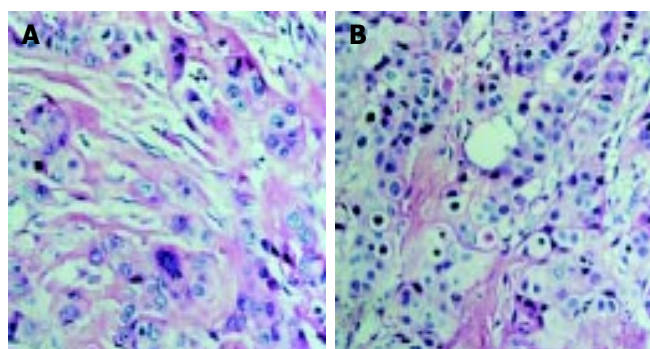
### Immunohistochemistry

With immunohistochemical staining, we examined the expression of survivin in pancreatic cancer. The results are shown in Figure 1. The expression of survivin was localized in cytoplasm of tumor cells, which are shown as brown granules in Figure 1 (SP), and in Figure 2 (HE). Survivin was expressed in 34 of 42 pancreatic cancer samples, but not expressed in the 10 samples from normal pancreatic tissue. The expression rate was 81.95%.

To study the relationship between the expressions of survivin and Bcl-2, 42 cases were analyzed. The results are shown in Table 2.



**Figure 1** Expression of survivin in poorly and well differentiated pancreatic cancer. A: Expression of survivin in poorly differentiated pancreatic cancer (SP×400). B: Expression of survivin in well differentiated pancreatic cancer (SP×200).



**Figure 2** Poorly and well differentiated pancreatic cancer. A: poorly differentiated pancreatic cancer (HE×400). B: well differentiated pancreatic cancer (HE×400).

**Table 1** Relationship between survivin expression in pancreatic cancer and clinicopathologic parameters

		Survivin expression		P	r
		(-,+)	(++,+++)		
Sex	Male	11	7	>0.05	
	Female	13	11		
Location	Head	16	14	>0.05	
	Body,Tail	8	4		
Differentiation	High	14	4	<0.05	0.361
	Low	10	14		
Distant metastasis	Present	8	8	>0.05	
	Absent	16	10		
Stage	I-II	18	8	<0.05	0.311
	III-IV	6	10		

**Table 2** Correlation between expressions of survivin and Bcl-2

	Survivin expression		P	r
	(+)	(-)		
Bcl-2 (+)	28	2	<0.01	0.499
Bcl-2 (-)	6	6		

## DISCUSSION

Survivin, a recently-characterized member of IAP family, was isolated from the human gene bank by Altieri *et al.* in 1997 using effector cell protease receptor-1(EPR-1) cDNA<sup>[6]</sup>. Recently, great progress has been made in the structure and function of survivin and its correlation to malignant tumors.

Many findings have suggested that survivin may express selectively in different tissues. Survivin was max expressed or poorly expressed in normal terminally-differentiated tissues, whereas it was extensively expressed in many kinds of human tumor tissues<sup>[7-14]</sup>.

Studies have shown that Caspase is responsible for apoptosis, which can activate in cascade and lyze protein, thus determining the pattern of apoptosis. Survivin could directly inhibit the activities of Caspase-3 and 7 and block the process of apoptosis and indirectly inhibit Caspase through P21. Therefore survivin would bind to cell cycle apoptosis factor CDK4 to form survivin-CDK4 complex, and then release of P21 from CDK4 complex. When P21 was bound to mitochondrial Caspase-3, it could inhibit its activity, thus preventing apoptosis<sup>[15-17]</sup>.

The results of our study showed that survivin was highly expressed in pancreatic tumor tissues, but not in normal pancreatic tissues, suggesting that it might play a critical role in the development and progression of pancreatic cancer. Furthermore, there was a positive correlation between survivin expression and tumor TNM staging and differentiation grade. It can be concluded that survivin expression is indicative of higher invasiveness or poor prognosis in pancreatic cancer.

Bcl-2 was the first characterized anti-apoptotic gene with an inhibiting apoptotic pathway different from that of survivin. Bcl-2 could regulate apoptosis by preventing cytochrome C release from mitochondrion to cytoplasm. Whereas, survivin acted by direct inhibition of the terminal effector proteases of apoptosis, i.e., Caspase-3 and Caspase-7.

Our study demonstrated that the expression of survivin was positively correlated with that of Bcl-2. It might be caused by the same transcription and activation mechanism of survivin and Bcl-2, or both might be regulated by GC-rich promoters, and were then transcribed and activated to enhance cell proliferation, acting synergistically to inhibit apoptosis<sup>[18-29]</sup>.

Asanuma *et al.*<sup>[2,5]</sup> investigated whether survivin expression could directly regulate cancer sensitivity to radiotherapy using gene-transduced pancreatic cancer cell strain (MIAPaCa-2). Their results showed that survivin expression could directly down-regulate pancreatic cancer sensitivity to radiotherapy.

In addition, Rohayem *et al.*<sup>[12,30,31]</sup> observed that specific anti-survivin antibody could be detected in serum of patients with cancers of the lung and colon, suggesting that this antibody could be used as a new diagnostic marker for cancers of the lung and colon. Furthermore, Smith *et al.*<sup>[32]</sup>, Moore *et al.*<sup>[33]</sup>, and Swana *et al.*<sup>[34]</sup> found that survivin levels in urine could be used to diagnose primary and recurrent bladder carcinomas, thus providing a new idea for the diagnosis of pancreatic cancer<sup>[35-39]</sup>.

In conclusion, Survivin can be used as a cancer therapeutic target because of its selective expression in the tissue concerned. Moreover, natural antisense nucleic acid for Survivin-endogenous EPR-1 has become a new hot issue for the tumor gene therapy.

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