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Expression of a novel apoptosis inhibitor-survivin in colorectal carcinoma

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Abstract

AIM: To investigate the role of survivin expression in the pathogenesis of colorectal carcinoma.

METHODS: Immunohistochemistry S-P method and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) were used to detect the expression of survivin and apoptotic cell *in situ* in colorectal cancerous tissues, para-cancerous tissues and normal tissues of 48 cases of colorectal carcinoma.

RESULTS: The survivin positive unit (PU) was higher in cancerous tissues (38.76±5.14) than in para-cancerous (25.17±7.26) or normal tissues (0.57±0.03) (P<0.05). The apoptosis index (AI) of para-cancerous tissues was (7.51±2.63%) higher than cancerous tissues (4.65±1.76%). The expression of survivin was associated with pathological grade, lymph node metastasis and Dukes stage of colorectal carcinoma.

CONCLUSION: Survivin expression may play an important role in carcinogenesis of colorectal carcinoma and may be associated with malignant biological behaviors of colorectal carcinoma.

Key words: Survivin; Colorectal carcinoma; Cell apoptosis

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INTRODUCTION

Survivin is a new member of inhibitors of apoptosis proteins (IAP) gene family that has been found recently. The survivin gene lies in the 17q25 of human chromosome with unique structure and characteristics, coding a 16.5 KD protein[1]. Survivin protein contains only one BIR (baculovirus IAP repeat) domain and does not have the zinc-binding fold terminated with carboxyl. Furthermore, under normal circumstances survivin is expressed in embryonic and fetal tissues, but completely downregulated in normal adult tissues. Interestingly, this protein is found to be prominently reexpressed in a variety of human malignant transformation cell lines and tumorous tissues[2,3]. And its unique structure and biological function have interested so many scholars during their researches on the molecular biology of tumors.

Colorectal carcinoma has a high incidence in China. The deficiency of cell apoptosis plays an important role in the pathogenesis of this carcinoma. It is still uncertain about the role of survivin expression in colorectal carcinoma. In the present study, the expression of survivin and cell apoptosis were detected. The correlation between survivin expression and cell apoptosis and the role of survivin in the pathogenesis of colorectal carcinoma were also investigated.

MATERIALS AND METHODS

Study materials

Forty-eight cases of colorectal carcinoma who received rectetomy were obtained from the Department of General Surgery, Renmin Hospital of Wuhan University. Twenty-six of them were male and 22 female. The mean age was 55.8 years ranged from 37 to 75 years. All the cases did not receive radiation treatment or chemotherapy before surgery and had been diagnosed by two doctors at the department of pathology. Three pieces of tissues were taken respectively from cancerous tissues, para-cancerous tissues (5 cm away from cancerous tissues) and normal tissues (10 cm away from cancerous tissues). All the specimens were fixed in 10% neutral-buffered formalin, dehydrated in ascending series of ethanol and routinely embedded in paraplast. Sections were cut at 4 μm, stained with hematoxylin and eosin for histopathological and immunohistochemical evaluation as well as TUNEL. The clinicopathological parameters are summarized in Table 1.

Immunohistochemical analysis

All the specimens were incubated in 3% hydrogen peroxide for 15 min to inactivate the endogenous peroxidase and then heated in 0.01 mol/L citrate buffer for antigen retrieval through 12 min microwave pre-treatment. Incubated with 10% goat serum, all the specimens were subsequently reacted with rabbit-anti-human survivin polyclonal antibody (Neomarkers, USA, 1:1 000 dilution) at 4 ℃ overnight.

Table 1 Relationship between survivin PU and pathological parameter in 48 cases with colorectal carcinoma (mean±SD)

Subjects	Clinical pathological index	*n*	Survivin PU	*P*
Gender	Male	26	42.95±16.87	*P*>0.05
	Female	22	30.96±16.06	
Age (yr)	<55	14	36.95±19.86	*P*>0.05
	⩾55	34	38.96±15.37	
Tumor	<3 cm	14	36.15±16.29	*P*>0.05
Diameter	⩾3 cm	34	38.28±18.16	
Pathological	High and intermedium differentiated	24	33.16±14.28	*P*<0.05
Grade	Cannular adenocarcinoma			
	Low differentiated tubular adenocarcinoma	10	51.93±20.89	
	Other types	14	35.61±17.22	
Lymph node	Negative	26	30.12±13.33	*P*<0.05
	Positive	22	56.21±11.95	
Dukes stage	A	16	20.16±5.16	*P*<0.05
	B	4	24.85±3.12	A / B Stage
	C	10	45.13±10.21	*VS*
	D	14	59.66±10.21	C / D stage

Immunohistological staining was performed according to SP detection kit.

TUNEL staining

Apoptotic cells were detected, according to the procedure recommended by *in situ* cell apoptosis detection kit (Boehringer Mannheim Company, Germany).

Evaluation criteria

Immunohistochemical quantitative evaluation All the analyses were performed using the HIPAS-2000 computer image analysis system (produced by Tongji Qianping Image Engineering Company) Images were captured at ×400 magnification by micrographic system . Image analysis system separated staining positive area from background. Then the gray level units as well as areas of positive staining and background could be measured. According to Shen's method, positive unit represents the relative concentration of positive staining[4]. Each section was observed randomly at five areas and the mean PU was calculated.

Evaluation criteria for cell apoptosis

The apoptotic cells were located sporadically with nuclei stained yellow or brownish yellow. A mean percentage of positive cells among 500 cells was determined in five areas at ×400 magnification. The results could be recorded as apoptosis index (AI).

Statistical analysis

All analyses were performed with *t* test and ANOVA using SPSS 9.0 software (Statistical Package for Social Science). *P* values<0.05 were considered to indicate statistical significance.

RESULTS

Survivin PU and apoptosis index of cancerous, paracancerous and normal tissues

Survivin PU were mainly in the cytoplasm of para-cancerous or cancer cells. The nuclei could be stained equally light yellow or brownish yellow, located sporadically or in the form of sheets (Figure 1A). Survivin PU was rarely expressed in normal large intestinal mucosa (Figure 1B). Quantitative analysis of immunohistochemistry is summarized in Table 2. The apoptotic cells were distributed sporadically or in clusters with brownish yellow nuclei. apoptotic cells were found to be rare and weak stained in normal large intestinal mucosa, most of which were located in epithelium (Figure 2A), but scattered sporadically in cancerous (Figure 2B) and para-cancerous tissues. Survivin PU in cancerous tissues 38.76±5.14 was significantly higher than in para-cancerous 25.17±7.26 and normal tissues 0.57±0.03 (*P*<0.05). And the AI in para-cancerous tissues, (7.51±2.63)% was significantly higher than in cancerous tissues, (4.65±1.76)%(*P* = 0.0075).

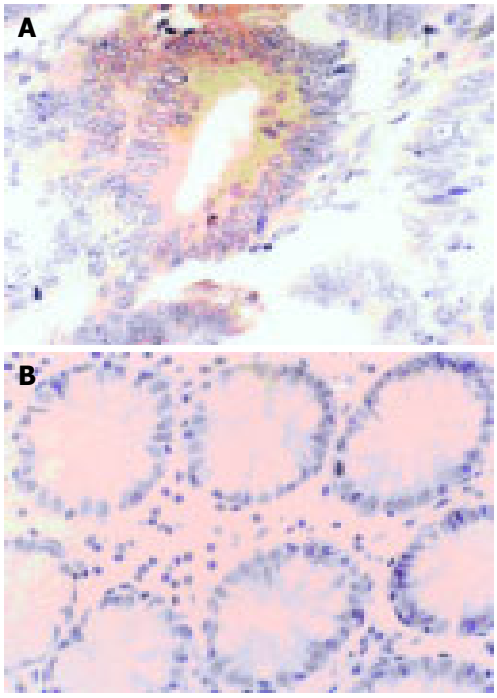

Figure 1 Expression of survivin in colorectal cancerous and normal tissues by S-P method. (x400). **A**: colorectal cancerous tissues; **B**: normal colorectal tissues.

Table 2 The comparison of survivin PU and AI in cancerous, paracancerous and normal tissues (mean±SD)

Subjects	Cancerous tissues	Paracancerous tissues	Normal tissues	*P*
Survivin PU	38.76±5.14	25.17±7.26[a]	0.57±0.03[a]	*P*
AI (%)	4.65±1.76	7.51±2.63	1.75±0.49	*P*

[a]*P*<0.05, Survivin PU of cancerous tissue *vs* paracancerous or normal tissue; *P* = 0.0075, AI of paracancerous tissue *vs* cancerous tissue.

Relationship between survivin PU and clinicopathological parameters

Survivin PU was not correlated with sex, age and tumor diameter of patients, but correlated with pathological grade, lymph node and Dukes stage. Survivin PU in cancerous tissues with low differentiation, lymph node positive and Dukes C/D stage was higher than in cancerous tissues with high differentiation, lymph node negative, and Dukes A/B stage.

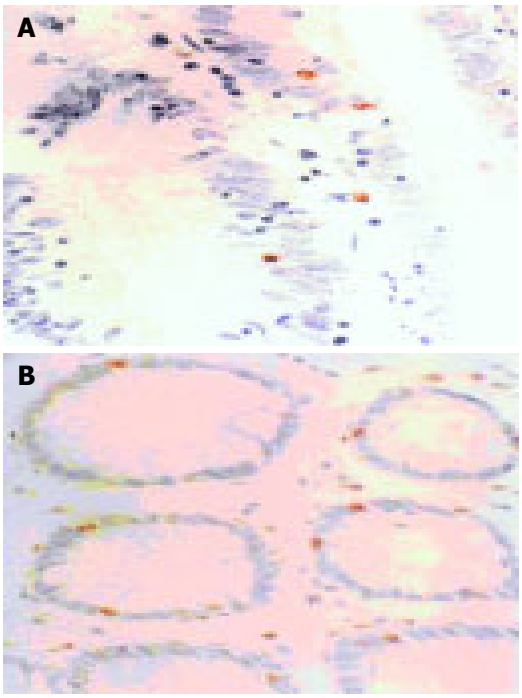

Figure 2 Apoptotic cells in normal colorectal epithelial and cancerous tissues by TUNEL method. (x400). **A**: normal colorectal epithelial tissues; **B**: colorectal epithelial tissues.

DISCUSSION

According to recent clinical and statistical data, there was a gradually increasing incidence in colorectal carcinoma. carcinogenesis can be regarded as a complex process with multi-gene participation and multi-steps. As we all know, most colorectal carcinomas originate from adenoma. Abnormalities in the control of programmed cell death (apoptosis) play an important role in the pathogenesis of colorectal carcinoma during the process from adenoma to cancer. It has been proved that the genes closely related with the control of colorectal cell apoptosis include bcl-2, c-myc, p53 and IAP gene family. So the research and application of those apoptosis-related genes is of great importance in the diagnosis, treatment and prognosis judgement of colorectal carcinoma.

Survivin is a new member of IAP gene family, which was obtained by Altieri in Yale University through hybridization and filtration of human genome[5]. It can combine with microtubules of mitotic spindle,and through interaction with caspases it can inhibit cell apoptosis[6]. Survivin affects various terminal effect factors and might be one of the strongest apoptosis inhibitory factors till now. This research using immunohistochemical staining showed that survivin was very weak in normal epithelial cells and partially expressed in para-cancerous tissues. But positive cells in para-cancerous tissues were less than in cancerous tissues with a weaker staining, suggesting that survivin expression could be an early event during the pathogenesis of colorectal carcinoma. This is in accordance with the researches on pancreatic and hepatocellular cancers. Evidence showed that survivin could be expressed in early stage of pancreatic cancer or prec-ancerous lesions[6,7]. Sarela found survivin mRNA mainly existed in survivin positive cancerous tissues, but hardly in survivin negative cancerous tissues[8]. Thus, it is proposed that, survivin is an oncogene, and also a marker with great potential for tumor diagnosis. Survivin antibody could also be taken as a common marker for early diagnosis of colorectal carcinoma[9,10].

This research proved that survivin PU had no significant correlation with sex, age, and tumor diameter, but correlated with differentiation stage, lymph node and Dukes stage of colorectal carcinoma. Survivin PU in cancerous tissues with low differentiation, lymph node positive and Dukes C/D stage was higher than in cancerous tissues with high differentiation, lymph node negative and Dukes A/B stage. It indicated that survivin PU was related with malignant biological behaviors. Its continuous expression might be associated with the development of malignant tumor. Researches on gastric carcinoma, lung cancer, breast cancer revealed that survivin PU was not only correlated with malignant biological behaviors such as invasion, metastasis, etc, but also correlated with recurrence, reduced survival time after surgery. It might be taken as an independent index for judging the prognosis[11-13]. Therefore, survivin is of great value for diagnosis and prognosis judgement of malignant tumors.

Survivin has the feature of selective expression in various malignant tumors, which might be essential for carcinogenesis of those tumors. But the mechanism for survivin to contribute to carcinogenesis of tumors is not clear yet. It is probably involved in cell apoptosis, proliferation, etc.[14]. Our research showed that the apoptotic cells mainly located in epithelium of normal large intestinal mucosa, but distributed sporadically in cancerous and para-cancerous tissues. AI of paracancerous tissues was higher than cancerous tissues, indicating that survivin could inhibit apoptosis of colorectal carcinoma cells. This might be regarded as part of mechanisms for its participation in carcinogenesis of colorectal carcinoma. But the precise pathway for survivin to inhibit apoptosis still needs further investigation. According to the present study, survivin had various pathways to inhibit apoptosis, which could also be found in other malignant tumors[15]. It might play an important role in the carcinogenesis of various tumors.

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