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World J Gastroenterol 2003;9(7):1435-1438
World Journal of Gastroenterology
 ISSN 1007-9327

• GASTRIC CANCER •

Expression of survivin in human gastric carcinoma and gastric carcinoma model of rats

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Received: 2003-01-04 **Accepted:** 2003-02-18

Abstract

AIM: To study the expression of survivin, an inhibitor of apoptosis protein, in human gastric carcinomas and gastric carcinoma models of rats.

METHODS: With the method of immunohistochemical staining, we studied the expression of survivin in 20 cases of chronic gastritis and 56 cases of gastric carcinomas. We used N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and high dose sodium-chloride diet to induce rat gastric carcinomas. Survivin expression was studied in glandular stomachs of normal rats, adenocarcinomas and tissues adjacent to the tumor, as well as in rats during the induction period.

RESULTS: Survivin was expressed in 27 of 56 (48.2 %) cases of human gastric carcinoma tissues and 1 of 20 (5 %) cases of chronic gastritis. It was found that the expression of survivin had no relation with the elements of age, tumor depth, tumor size, and disease stage, but was significantly related to histological type. The positive rate of survivin expression in cases of intestinal type was significantly higher than that in cases of diffuse type ($P<0.05$). In animal experiments, survivin expression in glandular stomachs of normal rats, of rats in middle induction period, in adenocarcinomas and tissues adjacent to tumor were 0, 40.0 %, 78.3 % and 38.9 %, respectively. Compared with the survivin expression in normal rats, the differences were significant.

CONCLUSION: These data imply that survivin plays an important role in the onset of gastric carcinoma and that high survivin expression is an early event of gastric carcinoma.

Zhu XD, Lin GJ, Qian LP, Chen ZQ. Expression of survivin in human gastric carcinoma and gastric carcinoma model of rats. *World J Gastroenterol* 2003; 9(7): 1435-1438
http://www.wjgnet.com/1007-9327/9/1435.asp

INTRODUCTION

Survivin, a member of the inhibitors of apoptosis protein (IAP) family, is a mitotic spindle-associated protein involved in linking mitotic spindle function to the activation of apoptosis in mammalian cells. The structure of full-length human survivin determined by X-ray crystallography is 2.7 A°. The structure forms a very unusual bow tie-shaped dimer. The unusual shape and dimensions of survivin suggest that it serves as an adaptor through its alpha-helical extensions[1]. Just like other IAP members, survivin can suppress apoptosis through combination with Caspase3, Caspase7 by baculoviral IAP repeat (BIR)[2,3]. The common pathway of apoptosis is the activation of Caspase3, Caspase7 or Caspase 6, hence high expression of survivin may protect cells from many apoptosis signals and help cells survive[4,5]. Now, substantial data have shown that inhibition of apoptosis plays a great role in carcinogenesis[6-10], so survivin may be an important factor in the development of cancer. It has reported that survivin is undetectable in terminally differentiated adult tissues and becomes prominently expressed in transformed cell lines and in most common human cancers of lung, colon, pancreas, prostate and breast[2]. Some data indicate that high expression of survivin is correlated with poor prognosis and chemotherapy resistance[11-13]. In this study, we investigated the expression of survivin in human gastric carcinoma and its relationship with clinicopathological factors, as well as the expression of survivin in gastric carcinoma models of rats.

MATERIALS AND METHODS

Patients and samples

A total of fifty-six cases of gastric carcinoma were involved in this study including 46 males and 10 females. The age range was 26-79 years, mean age was 59.8 years. The patients with gastric carcinoma, having undergone potentially curative tumor resection at Huashan Hospital from 2000 to 2001, had received neither chemotherapy nor radiation therapy before surgery. The histological types of tumors were classified according to Lauren as intestinal type and diffuse type, and the disease stage was defined in accordance with the tumor-node-metastasis (TNM) classification[14]. There were 32 cases of intestinal type and 24 cases of diffuse type. Materials were composed of 19 cases of stage I, 8 cases of stage II, and 29 cases of stage III. The tissues containing principal tumor were selected and fixed with formalin, embedded in paraffin routinely. Serial sections of 4 μm were prepared for immunohistochemical examination and histopathological study. The expression of survivin was investigated in these 56 gastric carcinoma patients and 20 cases of chronic gastritis by immunohistochemical examination.

Animal experiment

Forty-six male 6-week-old Wistar rats were divided into 2 groups. Thirty rats in group A were fed with a diet supplemented with 8 % NaCl for 20 weeks and simultaneously given N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in drinking water at a concentration of 100 ug/ml for the first 17 weeks. From week 18, these rats were given normal water. From week 21, these rats were fed with normal diet for 15 weeks. The other sixteen rats in group B were fed with normal diet for 35 weeks and served as the control. At week 20, 10 rats in group A were killed and all the rest animals were killed

at the end of week 35. The whole stomach and a part of duodenum were sampled and cut open along the greater curvature. The number of tumors with their locations and sizes were recorded in details. All the specimens were histopathologically investigated and the expression of survivin was examined with immunohistochemical analysis as they were done in human specimens.

Immunohistochemical staining for survivin and assessment of its expression

Anti-survivin polyclonal antibody was purchased from Santa Cruz Company. Immunohistochemical analysis was carried out with the standard streptavidin-biotin-peroxidase (SP) complex technique using the Ultra sensitive™ S-P kit (Maixin-Bio Company). One case of stage III gastric carcinoma intensively and reproducibly stained for survivin expression in more than 50 % of tumor cells served as positive control. Negative control slides were stained without primary antibody. To assess the expression of survivin in various samples examined, a 4-grade-method was established according to the mean percentage of positive tumor cells and their intensity. Moderately stained slides with a mean percentage of positive tumor cells no less than 30 % were scored as positive (+). Moderately or intensively stained slides with a mean percentage of positive tumor cells more than 70 % were scored as intensely positive (+++). Slightly or moderately stained slides with a mean percentage of positive tumor cells between 30 % and 70 % were scored as moderately positive (++). Slightly or moderately stained slides with a mean percentage of positive tumor cells less than 30 % were scored as negative (-).

Statistical analysis

Software Stata (version 6.0, STATA Corp, College Station) was applied to compare the rates.

RESULTS

Correlation between expression of survivin and clinicopathological factors in human gastric carcinomas

Positive staining for survivin was located in cytoplasm of tumor cells (Figure 1). A clinicopathological analysis of survivin-positive cases is shown in Table 1. The expression of survivin was positive in 27 of 56 (48.2 %) cases of gastric carcinoma tissues and 1 of 20 (5 %) cases of chronic gastritis. Expression of survivin had no relation with age, tumor depth, tumor size, and disease stage (P>0.05), but it was significantly related to histological type. The positive rate of survivin expression in cases of intestinal type was significantly higher than that in cases of diffuse type (P<0.05).

Table 1 Correlation between expression of survivin and clinicopathological factors in human gastric carcinomas

Variables	Cases	Survivin expression *n* (%)	*P*
Chronic gastritis	20	1(5%)	<0.001
Gastric carcinoma	56	27(48.2%)	
Age			
<55	18	11(61.1%)	0.184
>55	38	16(42.1%)	
Invasion size			
<12.3 cm^2	31	16(51.6%)	0.571
>12.3 cm^2	25	11(44.0%)	
Invasion depth			
Muscular layer unaffected	14	5(35.7%)	0.280
Muscular layer affected	42	22(52.4%)	
Lymph node metastasis			
Regional lymph Node unaffected	20	9(45.0%)	0.720
Regional lymph Node affected	36	18(50%)	
Histological type (Lauren) intestinal	32	20(62.5%)	0.013
Diffuse	24	7(29.2%)	
Disease stage(TMN)			
I	19	8(42.1%)	0.873
II	8	4(50.0%)	
III	29	15(51.7%)	

Notes: The disease stage of each tumor was defined in accordance with the tumor-node-metastasis (TNM) classification, the histological type was classified according to Lauren, and other pathological variables were defined in accordance with the Gastric Carcinoma[14].

The expression of survivin in gastric carcinoma models of rats

By the end of week 35, neoplastic foci were found in antral mucosa in 18 rats of group A which were histologically determined to be adenocarcinomas. Of these 18 rats, the total number of adenocarcinomas was 23, and 22 were very well differentiated, which could be classified as intestinal type according to Lauren classification. Just like human gastric carcinoma, positive staining for survivin was located in cytoplasm of tumor cells (Figure 2). No survivin expression was detected in antral mucosa of normal rats (group B). As shown in Table 2, in antral mucosa of those rats at week 20, survivin expression was positive in 4 of 10 rats. In adenocarcinomas and tissues adjacent to the tumor, the ratio was 78.3 % and 38.9 %, respectively. Compared to normal tissue, the difference was significant (Table 2).

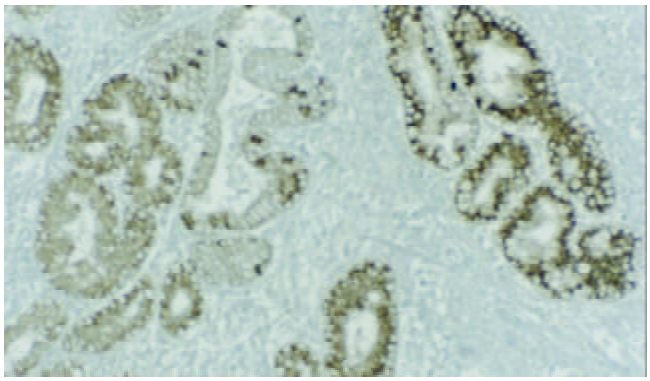

Figure 1 Immunohistochemical staining for the expression of survivin in human gastric carcinoma. Positive staining for survivin was located in cytoplasm (×50).

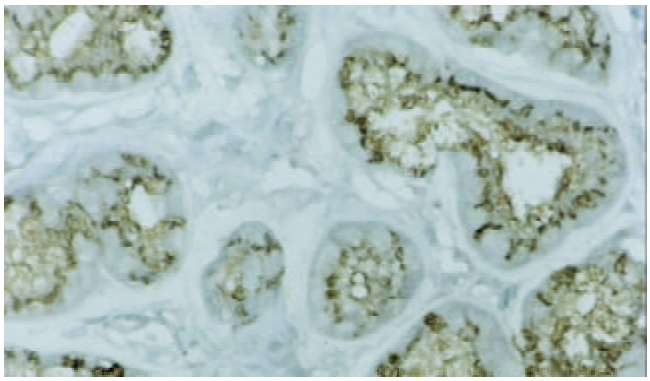

Figure 2 Immunohistochemical staining for the expression of survivin in gastric carcinoma of rat model. Positive staining for survivin was also located in cytoplasm. (×100).

Table 2 Survivin expression in experimental gastric carcinoma

	Cases	Survivin expression	Positive rate(%)
Control (group B)	16	0	0
Group A			
Antral mucosa of rats at week 20	10	4	40%[a]
Adenocarcinomas	23	18	78.3%[b]
Tissues adjacent to tumor	18	7	38.9%[c]

Ten rats of group A were killed at week 20, and the antral mucosa were examined. At week 35, all rats in both group A and B were killed and investigated. Tissues adjacent to tumor were defined as the tissues 5 mm away from the edge of tumor. [a]$P<0.05$ *vs* control group, [b]$P<0.001$ *vs* control group, [c]$P<0.01$ *vs* control group.

DISCUSSION

Survivin is expressed in a series of human cancers, and it has been widely accepted that survivin is highly related to the onset and development of cancer. In this study, we adopted human gastric carcinoma specimens and experimental gastric carcinoma models to discuss the effect of survivin on gastric cancer.

Survivin was expressed in 27 of 56 (48.2 %) cases of human gastric carcinoma tissues and only 1 of 20 (5 %) cases of chronic gastritis. In experimental gastric carcinoma, the positive rate rose to 78.3 %. These data suggest that high expression of survivin is a common phenomenon in gastric cancer and inhibition of apoptosis resulted from survivin expression may play an important role in carcinogenesis.

Our data on human gastric carcinoma proved that the expression of survivin in intestinal type cases is significantly higher than that in diffuse type cases and the expression of survivin is correlated with intestinal histological type. This result is consistent with Lu[15]. The Lauren histological classification has a specific epidemiological significance because the epidemiological study has determined that intestinal type cases are dominant in high risk areas while diffuse type cases are dominant in low risk areas. Substantial data suggest that intestinal type cases are highly related to circumstances and diffuse type cases are related to heredity. At present, Correa' s theory of an atrophy-metaplasia-dysplasia-carcinoma sequence in the development of intestinal type gastric carcinoma has been widely accepted[16-22]. The fact that the expression of survivin in intestinal type was higher than that in diffuse type suggests that survivin plays a more important role in the former and indicate that survivin may be an important factor contributing to the conversion from atrophy to carcinoma. Our animal experiment provided further evidences.

In our animal experiment, 95.7 % of the induced gastric carcinomas were intestinal type, and we did find atrophy and dysplasia lesions during the induction period. These data suggest that our rat model could simulate the development of human gastric carcinoma (intestinal type). We dynamically investigated the expression of survivivn during the induction period and found 4 cases were positive in 10 rats at week 20, and 78.3% was positive in gastric carcinoma. These data indicate a rising trend of survivin expression during the development of gastric cancer, and suggest an important role of survivin expression in tumor formation.

Some scholars concluded that survivin could only be found in tumor[15], but others thought it could be found in precancerous lesions[23,24]. Our results supported the latter. In our experiment, the expression of survivin was positive in some gastritis patients, and in some rats during the induction period, as well as in some tissues adjacent to tumor. Hence, we may conclude that survivin is not only expressed in cancer tissues but also in damaged tissues. The expression of survivin occurs before the formation of adenocarcinomas, and is an early event in carcinoma development.

Recently, some studies have partly explained why survivin was highly expressed in cancer. According to the study of Hoffman *et al*[25], the anti-apoptotic gene, survivin, is a p53-repressed gene. Chromatin immunoprecipitations indicate that wild type p53 binds survivin promoter *in vivo*, which results in transcriptional repression. Mirza *et al*[26] study implicated that wild-type p53 suppresses survivin expression at both mRNA and protein levels. It is widely accepted that mutated p53 loses its function as a tumor inhibitor and this may contribute to the loss of inhibition to survivin. Due to the high incidence of p53 mutation in gastric cancer, we put forward the hypothesis that long term effect of carcinogen should lead to p53 or other important gene damage, which results in survivin expression and apoptosis inhibition. Abnormal apoptosis leads to carcinogenesis.

According to our clinical data, age and prognostic factors such as different tumor size, tumor depth, lymph node metastasis or disease stage have no significant correlation with survivin expression, which indicates that survivin has little impact on tumor biological behavior.

Survivin plays an important role in gastric carcinoma and is a key molecule of cell cycle, mitosis and apoptosis[27]. Moreover, inhibition of survivin function will result in cell apoptosis[28-30]. The antisense oligonucleotide targeting survivin expression sensitizes lung cancer cells to chemotherapy[30]. All these data suggest that survivin may be an attractive target for gastric cancer therapy.

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Edited by Xu XQ and Wang XL