Expression of survivin protein in human colorectal carcinogenesis

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AIM: To identify the role of survivin in colorectal carcinogenesis and the relationship between Survivin and histological differentiation grade of colorectal carcinoma.

METHODS: Immunohistochemical staining of survivin by using the monoclonal antibody was performed by the standard streptavidin-peroxidase (SP) technique for the 188 paraffin sections which included 30 normal colorectal mucosas, 41 adenomas with low grade dysplasia, 30 adenomas with high grade dysplasia, and 87 colorectal carcinomas which were classified as high, middle and low differentiated subgroups which included 33, 28, 26 cases respectively.

RESULTS: Expression of survivin was observed in the cytoplasm of adenoma with dysplasia and colorectal carcinoma cells. No immunoreactivity of survivin was seen in normal mucosas. The positive rate of survivin increased in the transition from normal mucosas to adenomas with low grade dysplasia to high grade dysplasia/ carcinomas (0.0 %, 31.7 %, 56.7 % and 63.2 % respectively). But the difference between high grade dysplasia and carcinomas had no statistical significance. Positive rate was not related to histological differentiation grade of colorectal carcinoma. Moreover, there was no correlation between histological differentiation grade of colorectal carcinoma and immunoreactive intensity of survivin.

CONCLUSION: The expression of survivin is the essential event in the early stage of colorectal carcinogenesis and plays an important role in the transition sequence and it is not related to histological differentiation grade of colorectal carcinoma. It thus may provide a new diagnostic and therapeutic target in colorectal cancer.

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INTRODUCTION
Disturbance of apoptosis is thought to be very important in neoplastic transformation and progression. Several tumor suppressor genes and oncogenes, such as p53 and the bcl-2 family, are involved in regulation of cell apoptosis, which were thoroughly studied[1-12]. Moreover, a gene family of inhibitor of apoptosis (IAP) has been identified recently. Survivin, a novel member of IAP family, directly inhibits caspase-3 and -7 activity[13] or conjugates caspase-9[14], and regulates the cell cycle in the G2/M phase by interact with spindle microtubules[15]. Survivin shows markedly different tissue expression compared with other IAPs. It is present during embryonic and fetal development, but is downregulated in normal adult tissues. However, it becomes re-expressed in a variety of cancers[16-20]. The unique feature makes it attractive as a target for cancer therapy[21]. In this study, we sought to investigate the expression of survivin in normal colorectal mucosas, adenomas with low grade dysplasia, adenomas with high grade dysplasia, and colorectal carcinomas by immunohistochemical staining method in order to identify the role of survivin in colorectal carcinogenesis and the relationship between survivin and histological differentiation grade of colorectal carcinoma.
secondary antibody and streptavidin conjugated to horseradish peroxidase, respectively. After three rinses with PBS, the sections were incubated with diaminobenzidine substrate, then rinsed with distilled water and counterstained with hematoxylin.

**Scoring criteria**
The mean percentage of positive cells for the expression of survivin was determined in at least 5 areas at 400-fold magnification, and cases with less than 10% positively stained cells were defined as negative. Cases with 10 to 29% positively stained cells were defined as “+”, 30 to 59% as “++”, and 60% or more than 60% as “+++”. These scorings were performed in a blinded fashion.

**Statistical analysis**
The difference and correlation were analyzed by χ² test. A value of $P<0.05$ was considered statistically significant.

**RESULTS**
By immunohistochemical staining, we examined the expression of survivin in adenoma-carcinoma sequence. Representative results were shown in Figure 1. The expression of survivin was observed in the cytoplasm of the benign and malignant tumor cells, whereas not in normal tissues. As shown in Table 1, the positive rate for survivin expression increased gradually from normal colorectal mucosas to adenomas with low grade dysplasia, adenomas with high grade dysplasia, and carcinomas. The expression rates were 0.0%, 31.7%, 56.7%, and 63.2% respectively. Analyzed by χ² test, there were significant differences in the expressions of survivin between the normal mucosas group and any one of the other groups, between adenomas with low grade dysplasia and carcinomas, and between adenomas with low grade dysplasia and adenomas with high grade dysplasia ($P<0.05$), while there were no significant differences between adenomas with high grade dysplasia and carcinomas ($\chi^2=0.40, 0.5<P<0.75$).

**Figure 1** Immunohistochemical staining of survivin in adenoma-carcinoma sequence. The expression of survivin was observed in the cytoplasm of the benign and malignant tumor cells, whereas not in normal tissues. N: normal tissue. D: adenoma with dysplasia; C: carcinoma; 1: ×200 fold; 2: ×400 fold.
were no significant differences between adenomas with high dysplasia and adenomas with high grade dysplasia, while there was no relationship between the differentiation grade of colorectal carcinoma and the expression intensity ($P>0.75$).

Table 1: Expression of survivin in the colorectal carcinogenesis

| Lesion                     | n  | Expression intensity | Expression rate % |
|----------------------------|----|----------------------|-------------------|
| Normal mucosas             | 30 | 30 0 0 0 0           | 0.0               |
| Adenomas with low grade dysplasia | 41 | 28 7 5 0             | 31.7              |
| Adenomas with high grade dysplasia | 30 | 13 3 7 7             | 56.7              |
| Carcinomas                 | 87 | 32 8 20 27           | 63.2              |

To study the relationship between survivin and histological differentiation grade of colorectal carcinoma, 87 cases of colorectal carcinoma were classified to high, middle and low differentiated subgroups. The results were shown in Table 2. Analyzed by $\chi^2$ test, there were no significant differences in the expressions of survivin among the subgroups ($P>0.90$). Moreover, there was no relationship between the differentiation grade of colorectal carcinoma and the expression intensity ($P>0.75$).

Table 2: Correlation between the differentiation grade of colorectal carcinoma and the expression intensity of survivin

| Lesion                     | n  | Expression intensity | Expression rate % |
|----------------------------|----|----------------------|-------------------|
| High differentiation       | 33 | 12 3 10 8            | 63.6              |
| Middel differentiation     | 28 | 11 2 4 11            | 60.7              |
| Low differentiation        | 26 | 9 3 6 8             | 65.4              |
| Total                      | 87 | 32 8 20 27           | 63.2              |

DISCUSSION

The development of colorectal carcinoma proceeds through a series of genetic changes involving the activation of oncogenes and loss of tumor suppressor genes. During this process, a disturbance in the balance between cell proliferation and apoptosis may underlie neoplastic development. Previous investigations have well studied the role of p53 and bcl-2 family[1-12]. The IAPs is a widely expressed gene family of apoptosis inhibitors. Survivin, a novel and structurally unique member of the IAP gene family, is the strongest apoptosis inhibitor. A characteristic finding of survivin is that it is reexpressed in the most common human carcinomas. The targeting mechanism is unclear. In this study, we aimed to identify the role of survivin in colorectal carcinogenesis and the relationship between survivin and histological differentiation grade of colorectal carcinoma.

In our study, we demonstrated that survivin was not expressed in normal colorectal mucosas, which coincided with previous reports. The expression of survivin was localized in the cytoplasm of the adenoma and carcinoma cells, and the positive rate for survivin expression increased gradually from normal colorectal mucosas to adenomas with low grade dysplasia, adenomas with high grade dysplasia, and to carcinomas. The expression rates were 0.0 %, 31.7 %, 56.7 % and 63.2 % respectively. Analyzed by $\chi^2$ test, there were significant differences in the expressions of survivin between the normal mucosa group and any one of the other groups ($P<0.05$), between adenomas with low grade dysplasia and carcinomas ($P>0.05$), and between adenomas with low grade dysplasia and adenomas with high grade dysplasia, while there were no significant differences between adenomas with high grade dysplasia and carcinomas. We analyze the adenomas with high grade dysplasia and the carcinoma groups. The positive rates of them were 56.7 % and 63.2 % respectively, and the total number of cases were 30 and 87 respectively, then, the value of $\chi^2$ was 0.40 and $P$ was more than 0.5 but less than 0.75. Thus, we consider that there is no difference between them. This result has not been analyzed by other studies, but it coincides with the clinical practice that the treatment of adenomas with high grade dysplasia is similar to that of the carcinomas.

To study the relationship between survivin and histological differentiation grade of colorectal carcinoma, 87 cases of colorectal carcinoma were classified to high, middle and low differentiated subgroups. The number of cases were 33, 28, and 26 respectively, and the positive rates were 63.6 %, 60.7 % and 65.4 % respectively. Analyzed by $\chi^2$ test, there was no significant difference in the expressions of survivin among the subgroups ($P>0.90$). Moreover, there was no relationship between the differentiation grade of colorectal carcinoma and the expression intensity ($P>0.75$). These results conflict with those of Wang Mei et al[22], who thought the expression of survivin was related to the tumor histological grade in cervical carcinoma. These may be due to the different tissue origin. There was no significant difference in the expressions of survivin among the different grade of colorectal carcinoma, which makes it possible to use survivin as a tumor-specific target for therapy or diagnosis. That is, no matter what historical grade the colorectal carcinoma is, the cancer can be diagnosed by detecting survivin and be treated by targeting survivin. Meanwhile, the normal cells will not be killed because survivin is not expressed in normal cells. Survivin is an attractive candidate for cancer therapy. Therefore, our study gives some directions to diagnose and treat colorectal cancer. Whether the expression of survivin can predict prognosis in cancer or not is still under discussion[23-36].

Survivin, a novel mammalian IAP molecule, has interested scholars for its unique developmentally regulated expression and mechanism. There are still many problems to be solved, such as the molecular mechanism for its selective expression, the details about its anti-apoptosis, and so on. The targeting therapy is just beginning. Therefore, it is worthy to be further studied to settle a firm basis of tumor diagnosis and therapy.

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