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RAPID COMMUNICATION

Study of the expressions of *p*53 and *bcl*-2 genes, the telomerase activity and apoptosis in GIST patients

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Abstract

AIM: To explore the relationship between clinicobiological behavior and the expression levels of telomerase activity, apoptosis, *p*53 gene and *bcl*-2 gene in gastrointestinal stromal tumors (GISTs).

METHODS: The intensity of telomerase activity, apoptosis, *p*53 and *bcl*-2 expression in GISTs were detected by telomeric repeat amplification protocol, *in situ* end-labeling technique, and immunohistochemistry, respectively.

RESULTS: The positive rates of telomerase activity of malignant GIST, potential malignant GIST and benign GIST were 85% (17/20), 22.8% (2/9) and 0 (0/9), respectively. The apoptosis indices of malignant GIST, potential malignant GIST, and benign GIST were 11.7 ± 5.4, 30.2 ± 5.6 and 45.2 ± 7.2, respectively. The intensity of telomerase activity and apoptosis were related to the biological characteristics of GISTs (85% *vs* 22.8%, 0, 0; $P < 0.01$ or 11.7 ± 5.4 *vs* 30.2 ± 5.6, 45.2 ± 7.2, 72.1 ± 9.3; $P < 0.05$). The intensity of telomerase activity was negatively correlated with cellular apoptosis (22.9 ± 8.4 *vs* 9.5 ± 5.7, $P < 0.01$). The intensity of telomerase activity was positively correlated with *p*53, *bcl*-2 expression (40.0% *vs* 78.9%, 40.0% *vs* 84.2%; $P < 0.05$).

CONCLUSION: The detection of telomerase activity, apoptosis and its control genes in GIST will be helpful for the discrimination of the malignant and benign GIST and evaluation of the prognosis.

Key words: Gastrointestinal stromal tumors; Telomerase; *p*53; *bcl*-2; Apoptosis

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INTRODUCTION

Gastrointestinal stromal tumor (GIST) was first outlined by Mazur *et al*[1] in 1983. It was considered to originate from gastrointestinal submucous (SM) and muscular propria (MP) lamina, which is constructed by spindle cell and epithelial cell. GIST is classified into four phenotypes according to differentiation and immunohistochemistry, namely muscular type, neural type, mixed type and indeterminate type. The diagnosis of GIST is determined by pathological examination and the detection of certain immunohistochemical factors with special characteristics, such as CD117 or C-kit and CD34. Investigations on GIST have increased greatly these years[1-7]. This study aimed to explore the relationship between clinicobiological behavior and the expression levels of telomerase activity, apoptosis, *p*53 gene and *bcl*-2 gene in GISTs.

MATERIALS AND METHODS

Subjects

A total of 38 GIST patients (23 men and 15 women, with mean age of 52.6 years), who underwent surgical resection from 1996 to 2006 in the 2nd Affiliated Hospital of China Medical University, were enrolled in this study. The locations of the tumors were in the stomach ($n = 17$, 44.7%), small intestine ($n = 11$, 28.9%), colon and rectum ($n = 10$, 26.4%). The immunohistochemical factors CD117 and CD34 were detected in the specimens. According to the classification of Ji *et al*[8], the 38 GIST specimens were classified into benign GIST ($n = 9$, 23.7%), potential malignant GIST ($n = 9$, 23.7%), and malignant GIST ($n = 20$, 52.6%). Another 10 specimens of normal smooth muscle tissue (NSMT) were selected as control group, 5 of which were from the stomach and the others were from the intestine. Each specimen was divided in two parts, one of which was stored at -80℃, and the others were embedded in paraffin blocks and cut into 5-μm thick sections.

Telomerase activity detection

According to manual, 30-50 mg tissue was sheared into small particles. After schizolysis and centrifugation,

Table 1 The expression level of telomerase and AI in tissue NSMT and GIST

Patients	*n*	Negative telomerase *n* (%)	AI (mean ± SD)
NSMT	10	-	72.1 ± 9.3
Benign GIST	9	-	45.2 ± 7.2
Potential malignant GIST	9	2 (22.8)	30.2 ± 5.6
Malignant GIST	20	17 (85.0)	11.7 ± 5.4

Table 2 Relationship between telomerase activity, AI, expression level of *p*53 and *bcl*-2 in GIST tissues (*n* = 9)

Telomerase group	AI (mean ± SD)	*p*53 (-) rate	*bcl*-2 (-) rate
Negative	9.5 ± 5.7[b]	78.9%[a]	84.2%[a]
Positive	22.9 ± 8.4	40.0%	40.0%

[a]$P < 0.05$, [b]$P < 0.01$ *vs* Negative group.

the supernatant was kept as templates in the following PCR amplification. Twenty-five microliters of TRAP mixture, 0.5 μL of reverse primer (primer sequence: 5'-AATCCGTCGAGCAGAGTT-3'), 0.2 μL of Taq polymerase and 1 μL of supernatant were mixed and kept at 30℃ for 30 min as the PCA reaction system. Amplification was carried out with a Perkin-Elmer DNA Thermal Cycler 480. The conditions for amplification were 94℃ for 4 min, followed by 30 amplification cycles at 94℃ for 30 s, 35℃ for 30 s, and 72℃ for 30 s. The 30 cycles were followed by a single extension cycle at 72℃ for 10 min. PCR products were electrophoresed on 120 mg/L polyacrylamide gel stained by silver nitrate. "scaliform" zone appeared in the telomerase positive result.

Cell apoptosis detection

Tumor tissue sections were stained with terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) to identify apoptotic cells. Briefly, the slide was firstly processed with 0.1 g/L polylysine, the sections were deparaffinized and rehydrated before treating with proteinase K (20 mg/L in 10 mmol/L Tris, pH 8.0) at 37℃ for 15 min and washed with 1 × TBS (20 mmol/L Tris, pH 7.6, 140 mmol/L NaCl). After inactivation of endogenous peroxidase, sections were rinsed in TdT buffer (30 mmol/L Tris, 140 mmol/L sodium cacodylate, 1 mmol/L cobalt chloride, pH 7.2) and incubated with TdT at a 1:50 dilution and biotinylated dUTP at a 1:50 dilution in TdT buffer for 60 min at room temperature. The visualization for reaction products was performed with DAB for 10 min at room temperature, counterstained with hematoxylin. Specific positive tissue sections were used for negative controls by replacing the TdT with PBS in the reaction mixture. The positive staining was defined as light yellow to brown stain in the cytoplasm or nuclei. We selected 10 visual fields in each slide, and counted 1000 cells in every visual field. The apoptotic index (AI) = (total number of positive cells/1000) × 100%.

Detection of p53 gene and Bcl-2 gene

Immunohistochemical staining of *p*53, *bcl*-2 was performed with a standard avidin-biotin-peroxidase complex detection kit according to the manufacturer's instructions. *p*53 was located in the nuclei, while *bcl*-2 was located in the cytoplasm as light yellow to brown color. The expression was considered positive if the number of stained cells > 20% of the total cells in a slide.

Statistical analysis

Data were analyzed using the χ^2 test and Student's *t* test, when appropriate. $P < 0.05$ was considered statistically significant.

RESULTS

The expression level of telomerase in the specimens from malignant GIST patients were significantly higher than those from potential malignant GIST patients, benign GIST patients and normal smooth muscle tissue (NSMT) ($P < 0.01$). AI was found to be gradually decreased in the specimens from NSMT, benign GIST, potential malignant GIST and malignant GIST patients ($P < 0.05$) (Table 1).

The AI in the telomerase-positive group was significantly lower than that in the telomerase-negative group ($P < 0.01$). The expression levels of *p*53 and *bcl*-2 in the telomerase-positive group were both higher than those in the telomerase-negative group ($P < 0.05$) (Table 2).

DISCUSSION

Telomerase is a unique rib nucleoprotein that can synthesize telomeric DNA onto chromosomal ends as a template to compensate for the loss of telomeric repeats caused by the so-called "end-replication" problem[9-13]. A recent study demonstrated that most of malignant tumors showed telomerase activity[14-18], while normal somatic tissues and benign tumors were deprived of this property, except some germinal cells, hematopoietic stem cells and greminative layer cells[19-23]. The characteristics of the telomerase described above make it to be a significant index in cancer diagnosis and treatment than any other tumor markers used before. Few studies were carried on the relationship between the telomerase and GIST. In our study, 17 of 20 (85%) malignant GIST specimens were found to be telomerase-positive, which is in agreement with Shay *et al*[15]. It was suggested that telomerase would be an ideal marker for the diagnosis of malignant GIST. There were three malignant GIST specimens found to be telomerase-negative. It could not be excluded that the negative results were caused by the "telomerase para pathway" mechanism or the insufficient retraction of telomere which could not activate the expression of telomerase[16,17]. Two of nine potential malignant GIST specimens were also detected telomerase-positive. After necessary repeated trials, the consistent positive results excluded the possibility of false-positive result. These two patients should be followed for further malignant canceration. The results of our study showed that the detection of telomerase activity might be an artifice to discriminate the malignant and benign GIST.

Apoptosis, also called as programmed cell death, is

characterized by the generation of fragmented nuclei with highly condensed chromatin, protrusion of cytoplasm, and formation of apoptotic bodies. It is a process of cell-killing mechanism to keep homeostasis through the control of gene in physiological or pathological condition. Apoptosis plays an important role in keeping the normal conformation and function of tissues and organs. If certain gene mutations cause the apoptosis regulator decreasing, the cell mutated will survive and hyperplasia resulting in tumor formation will happen. Some researchers[24-30] have determined that apoptosis is tightly related to the development of tumors. Our study showed that there was significant negative correlation between apoptosis and the degree of GIST differentiation.

The telomere decurtates along with the mitosis. But when the telomere decurtates to some extent, apoptosis will occur due to the loss of the ability of mitosis. On the other hand, the telomerase in the cell might be activated, which would make the function of telomere recovered[31]. In our study, a remarkably negative correlation was observed between the telomerase activity of GIST and apoptosis. Moreover, a positive correlation was found between the telomerase activity and the expression level of *p*53 and *bcl*-2 genes. Our conclusion is consistent to some previous reports[17,21], while opposite to others[22,23].

In summary, the detection of telomerase activity, apoptosis and its control genes in GIST will be helpful for the discrimination of the malignant and benign GIST and evaluation of the prognosis. It would be a potential method for screening of a suitable therapeutic regimen specific to GIST to make telomerase or apoptosis as a curative target. More work should be done for further research on it.

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