PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 21; 12(3): 465-467
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Telomerase activity and human telomerase reverse transcriptase expression in colorectal carcinoma

Jian-Lun Liu, Lian-Ying Ge, Gui-Nian Zhang

Jian-Lun Liu, Department of Gastrointestinal and Breast Surgery, the Affiliated Cancer Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China
Lian-Ying Ge, Department of Endoscope, the Affiliated Cancer Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China
Gui-Nian Zhang, Department of Gastrointestinal Surgery, Guangxi Zhuang Autonomous Region Hospital, Nanning 530021, Guangxi Zhuang Autonomous Region, China
Supported by the Science Foundation of Health Bureau of Guangxi Zhuang Autonomous Region, No. 9954
Correspondence to: Jian-Lun Liu, Department of Gastrointestinal and Breast Surgery, the Affiliated Cancer Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China. jianlun@public.nn.gx.cn
Telephone: +86-771-5310708 Fax:+86-771-531200
Received: 2005-05-13 Accepted: 2005-06-11

Abstract

AIM: To study the activity of telomerase and the expression of human telomerase reverse transcriptase (hTERT) in colorectal carcinoma and its adjacent tissues, normal mucosa and adenomatoid polyp, and to evaluate their relation with carcinogenesis and progression of colorectal carcinoma.

METHODS: Telomerase activity and hTERT expression were determined in 30 samples of colorectal carcinoma and its adjacent tissues, normal mucosa and 20 samples of adenomatoid polyp by modified telomeric repeat amplification protocol (TRAP), enzyme-linked immunosorbent assay (ELISA) and immunohistochemical method.

RESULTS: Telomerase activity and hTERT expression were 83.33% (25/30) and 76.67% (23/30) respectively in colorectal carcinoma, which were obviously higher than those in paracancerous tissues (13.33%, 16.67%), normal mucosa (3.33%, 3.33%) and adenomatoid polyp (10%, 10%). There was a significant difference between colorectal carcinoma and other tissues (P=0.027). The telomerase activity and hTERT expression were higher in colorectal carcinoma with lymphatic metastasis than in that without lymphatic metastasis (P=0.034). When the histological classification and clinical stage were greater, the telomerase activity and hTERT expression increased, but there was no significant difference between them. In colorectal carcinoma, the telomerase activity was correlated with hTERT expression (positive *vs* negative expression of telomerase activity and hTERT, P=0.021).

CONCLUSION: Telomerase activity is closely correlated with the occurrence, development and metastasis of colorectal carcinoma. Overexpression of hTERT may play a critical role in the regulation of telomerase activity.

Key words: Colorectal carcinoma; Telomerase activity; hTERT expression

Liu JL, Ge LY, Zhang GN. Telomerase activity and human telomerase reverse transcriptase expression in colorectal carcinoma. *World J Gastroenterol* 2006; 12(3): 465-467

http://www.wjgnet.com/1007-9327/12/465.asp

INTRODUCTION

Colorectal carcinoma is one of the most common malignant tumors in digestive system, threatening human life and health. Recent investigations demonstrated that the telomerase activity is significantly increased in human malignant tumors, but is not expressed in normal somatic cells[1,2], suggesting there is a close relation between telomerase activity and malignant tumors. In order to study the role of telomerase activity in the carcinogenesis and progression of colorectal carcinoma as well as the correlation between telomerase activity and hTERT expression, the telomerase activity and hTERT expression were determined in colorectal carcinoma and its adjacent tissues, normal mucosa and adenomatoid polyp in this study.

MATERIALS AND METHODS

Patients and specimens

Thirty specimens of colorectal carcinoma and corresponding paracancerous tissues and normal mucosa were obtained by surgical resection, 20 specimens of adenomatoid polyp were taken during endoscopic examination. All patients were diagnosed pathologically and no patient received radiotherapy or chemotherapy before the sampling. Clinical staging showed Dukes' A in none, Dukes' B in 2 cases, Dukes' C in 24 cases, and Dukes' D in 4 cases. Well-differentiated adenocarcinoma

Table 1 Telomerase activity and hTERT expression in four different groups

Group	*n*	Telomerase *n* (%)	hTERT *n* (%)
Colorectal carcinoma	30	25 (83.33)[a]	23 (76.67)[a]
Adjacent peritumoral tissues	30	4 (13.33)	5 (16.67)
Adenomatoid polyp	20	2 (10.00)	2 (10.00)
Normal mucosa	30	1 (3.33)	1 (3.33)

[a]$P<0.05$ ($\chi^2=59.58$, $\chi^2=49.23$) *vs* adjacent peritumoral tissues, adenomatoid polyp and normal mucosa.

Table 2 Relation between telomerase activity and hTERT expression and clinical pathologic factors of colorectal carcinoma

Group	*n*	Telomerase (%)	hTRET (%)
Dukes' A	0	0	0
Dukes' B	2	1/2[a]	1/2[a]
Dukes' C	24	83.33 (20/24)	83.33 (20/24)
Dukes' D	4	4/4[a]	2/4[a]
High differentiation	5	2/5[a]	3/5[a]
Moderate differentiation	8	6/8[a]	5/8[a]
Poor differentiation	17	100.00 (17/17)	88.24 (15/17)
With lymphatic metastasis	10	80.00 (8/10)[b]	70.00 (7/10)[b]
Without lymphatic metastasis	20	0.00 (0/20)	5.00 (1/20)

[a]indicates cases less than 10 not included in the percentage; [b]$P<0.01$ *vs* the group without lymphatic metastasis

was found in 5 cases, moderately-differentiated adenocarcinoma in 8 cases and poorly-differentiated adenocarcinoma in 17 cases. Ten patients had lymph node involvement and 20 had no lymph node involvement. Of the patients with adenomatoid polyp, two had accompanying moderate-severe atypical hyperplasia. Specimens were collected within 30 minutes *in vitro*. Each specimen was divided into two parts, one for pathological diagnosis and immunohistochemical staining and the other for telomerase activity assay.

TRAP PCR ELISA protocol for telomerase activity assay

Telomerase activity was detected with a kit (Roche, Germany). Primers TS (5' AATCCGTCGAGCAGAGTT) and CX (5' CCCTTACCCTTACCCTTACCCTAA) were designed. Thirty cycles of PCR were performed at 25℃ for 30 min, at 94℃ for 5 min, at 94℃ for 30s, at 50℃ for 30s, at 72℃ for 90s, and a final extension at 72℃ for 10 min. The PCR products were analyzed and defined positive when $A>0.2$ on the reading of the microplate reader.

hTERT expression detection by immunohistochemical staining

Expression of hTERT was detected by immunohistochemical assay with the kit provided by Beijing Zhongshan Golden Biotechnology Co. Ltd according to the manufacturer's instructions. S-P method was adopted and positive calls were calculated and compared according to the accessory tester of the product. A negative control was prepared for each sample using PBS as the primary antibody. Microscopically, no staining was negative, karyon and perikaryon cytoplasms with brown granules were defined as positive cells.

Table 3 Correlation between telomerase activity and hTERT expression

		hTERT		
Telomerase	*n*	+	--	*P*
+	25	20	5	<0.05
--	5	3	2	

Statistical analysis

Statistical analyses were carried out with PEMS statistical-software. Chi-square test or Fisher's exact test was used for data processing. $P<0.05$ was considered statistically significant.

RESULTS

Telomerase activity and hTERT expression in colorectal carcinoma and its adjacent tissues, normal mucosa and adenomatoid polyp

The high telomerase activity and hTERT expression were found in 25 (83.33%) and 23 (76.67%) out of the 30 specimens of colorectal carcinoma, but in only 4 (13.33%) and 5 (16.67%) specimens of adjacent peritumor tissues, in 1 (3.33%) and 1 (3.33%) specimens of normal mucosa, in 2 (10.00%) and 2 (10.00%) out of the 20 specimens of adenomatoid polyp (Table 1).

Relation between telomerase activities and hTERT expression and clinical pathologic factors of colorectal carcinoma

The telomerase activity and hTERT expression had a close relation with lymphatic metastasis, and the positive expression rate in the patients with lymphatic metastasis was significantly higher than that in the patients without lymphatic metastasis ($P<0.05$). The telomerase activity and hTERT expression increased when the histological grade and clinical staging were greater, but the difference was of no statistical significance ($P>0.05$, Table 2).

Relation between telomerase activity and hTERT expression

The expression coincidence rate of telomerase activity and hTERT expression in colorectal carcinoma and its adjacent tissues, normal mucosa and adenomatoid polyp was 92% (23/25), 80% (4/5), 100%(1/1), 100%(2/2) respectively. The total coincidence rate of telomerase activity and hTERT expression was 90.91% (30/33). Of the 30 cases of colorectal carcinoma, 20 cases had positive expression in telomerase activity and hTERT expression, 2 showed no telomerase activity and hTERT expression. The conformity rate was 73.33% (22/30) ($P<0.05$, Table 3).

DISCUSSION

Telomerase is a ribonucleoprotein complex (a cellular

reverse transcriptase) consisting of three components: human telomerase RNA (hTR), telomerase-associated protein 1 (TP1/TLP) and human telomerase reverse transcriptase (hTERT). The first two components are expressed constitutively in both normal and tumor tissues and their expression levels are not correlated with the telomerase activity, whereas hTERT expression is closely correlated with telomerase activity in cells and tissues. Telomerase uses its internal RNA component as a template to synthesize telomeric DNA (TTAGGG)n, participating in the maintenance of telomere length and immortalization of cells[3]. Shay *et al*[4] reported that the total positive rate of telomerase activity was 85% in more than 20 malignant tumors and merely 9% in adjacent peritumor tissues and normal tissues, suggesting that telomerase activity is closely associated with malignancies. Our study showed that the telomerase activity and hTERT expression in colorectal carcinoma (83.33% and 76.67% respectively) were much higher than those in adjacent peritumoral tissues, normal mucosa and adenomatoid polyp. The telomerase activity and hTERT expression were positive in 2 cases of adenomatoid polyp with a diameter >2 cm, suggesting that telomerase activity may be an important index of canceration. This result is consistent with the findings of Tang *et al*[5]. The telomerase activity was higher in colorectal carcinoma with lymphatic metastasis than in that without lymphatic metastasis ($P<0.01$), indicating that the level of telomerase activity is closely related with lymphatic metastasis. The increased telomerase activity was in accord with the histological grade and staging of tumor, suggesting that telomerase activity plays a key role in the occurrence and development of colorectal carcinoma, and can be used as a marker for the early diagnosis and prognostic estimation of colorectal carcinoma.

hTERT is a telomerase reverse transcriptase isoform which is highly expressed in cell lines of positive telomerase[4]. hTERT transcription translated with hTR is indicative of telomerase activity *in vitro*. The activity of telomerase decreases or even disappears if the amino acid in hTERT is changed, whereas introduction of hTERT into normal cells activates the telomerase and prolongs cell life span while the karyotype and phenotype of cells remain normal[6-9]. In our study, the expression of hTERT was closely associated with telomerase activity, the coincidence rate of telomerase activity and hTERT expression was as high as 90.91% (30/33), suggesting that telomerase activity is related with hTERT expression. The results support the opinion that hTERT is an importent rate-limiting determinant of telomerase activity and the expression level of hTERT is directly associated with the telomerase activity. Since hTERT test can be conducted in paraffin- embedded tissues, more samples are available for telomerase activity test and hTERT may be used as a new tumor marker.

In conclusion, telomerase activity and hTERT expression in colorectal carcinoma are closely related. hTERT may play an important role both in the activation of telomerase and in the formation and development of colorectal carcinoma. hTERT can be used as a new marker for the early diagnosis of colorectal carcinoma.

REFERENCES

1 **Ramakrishnan S**, Eppenberger U, Mueller H, Shinkai Y, Narayanan R. Expression profile of the putative catalytic subunit of the telomerase gene. *Cancer Res* 1998; **58**: 622-625

2 **Urquidi V**, Tarin D, Goodison S. Role of telomerase in cell senescence and oncogenesis. *Annu Rev Med* 2000; **51**: 65-79

3 **Meyerson M**, Counter CM, Eaton EN, Ellisen LW, Steiner P, Caddle SD, Ziaugra L, Beijersbergen RL, Davidoff MJ, Liu Q, Bacchetti S, Haber DA, Weinberg RA. hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 1997; **90**: 785-795

4 **Shay JW**, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer* 1997; **33**: 787-791

5 **Tang R**, Cheng AJ, Wang JY, Wang TC. Close correlation between telomerase expression and adenomatous polyp progression in multistep colorectal carcinogenesis. *Cancer Res* 1998; **58**: 4052-4054

6 **Weinrich SL**, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtsteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB, Morin GB. Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. *Nat Genet* 1997; **17**: 498-502

7 **Beattie TL**, Zhou W, Robinson MO, Harrington L. Reconstitution of human telomerase activity in vitro. *Curr Biol* 1998; **8**: 177-180

8 **Nakayama J**, Tahara H, Tahara E, Saito M, Ito K, Nakamura H, Nakanishi T, Tahara E, Ide T, Ishikawa F. Telomerase activation by hTRT in human normal fibroblasts and hepatocellular carcinomas. *Nat Genet* 1998; **18**: 65-68

9 **Bodnar AG**, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998; **279**: 349-352

S- Editor Wang XL **L- Editor** Elsevier HK **E- Editor** Kong LH