TELOMERASE ACTIVITY IN HUMAN GASTRIC AND COLORECTAL CANCER AND SURROUNDING TISSUES

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Abstract

Objective: To study the telomerase activities in human gastric and colorectal tumors. Methods: The telomerase activity was assayed by the telomeric repeat amplification protocol (TRAP) technique. Forty human tumor samples including 9 colonic, 20 rectal and 11 gastric carcinomas and their surrounding tissues were used for the detection. Results: Thirty-six out of 40 human tumor samples exhibited telomerase activity regardless of the stages or the differentiation of the tumors. However, only 1 out of 39 tumor surrounding tissues showed telomerase activity. Conclusion: Telomerase may be a good diagnosis biomarker for tumor detection.

Key words: Stomach neoplasms, Colonic neoplasms, Rectal neoplasms, Telomerase, Tumor biomarkers.

Telomerase is the enzyme that synthesizes telomere (hexameric, 5'-TTAGGG-3`, repeats to the ends of mammalian chromosomal DNA). Morin[1] first reported that the activity of telomerase could be detected in the cultured Hela cells. Counter[2] found that late-stage ovarian carcinoma tumor cells maintain short stable telomere both *in vitro* and *in vivo* and that the telomerase activity is specifically activated in tumor cells, but not in normal somatic cells. Recently, increasing evidence shows that the telomerase activity may be associated with the development of the cancer cells. The phenomena have been examined more detail since the very sensitive assay method, the telomeric repeat amplification protocol (TRAP) technique has been developed by Kim[3] and has been commercially available. Using this TRAP methods, we were able to detect the telomerase activities in human tumor samples from 40 cancer patients. Comparisons of the telomerase activities of the human tumor tissues and the tissues from the tumor-adjacent areas of those patients were also conducted. The objective of this study is to confirm the hypothesis that telomerase activity can be detected in human tumor cells and to predict the possibility of telomerase in tumor diagnosis.

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MATERIALS AND METHODS

Reagents

TRAP-eze telomerase detection kit was purchased from Oncor Company. The major components of the CHAPS lysis buffer, such as EGTA, PMSF and CHAPS were from Sigma Chemical Co., Taq DNA polymerase was purchased from Promega Company. PBR 322 DNA/Hae II Markers were purchased from Gene Company.

Specimens

Human tumor and tumor-surrounding samples and nasopharyngeal cancer cell line (CNE_2) were kindly provided by Cancer Center of Sun Yat-Sen University of Medical Sciences. All the tissues were pathologically verified.

Extraction and Quantitation

The extraction of cellular protein from tumor or

tumor-surrounding tissues was performed according to the method of Kim.[3] The protein concentration were determined by Coomassie G250 assay.

Telomerase Activity Assay

Telomerase is a RNA-contained nucleoprotein which is sensitive to RNase. Therefore, sterilized glassware and supplies were used during the whole procedures. The protein extraction and the PCR reactions should be performed on the different area of the bench in order to avoid any possible cross contamination. The procedures were according to the detection kit with slightly modification in which two steps PCR cycles of 94°C/30 sec, 60°C/30 sec was changed to three steps cycles of 94°C/30 sec, 50°C/30 sec, and 72°C/90 sec and followed by an extension of 72°C for 10 min. The PCR products were then separated by using a 12.5% polyacrylamide gel electrophoresis (125v for 4 h). The gel was stained by silver nitrate technique and the results were observed by eyes.

Statistic Analysis

Results between two groups were compared using Chi-square analysis of SSPS software. Differences were considered statistically significant when $P<0.05$.

RESULTS

Two methods can be used for analyzing the activity of telomerase when using TRAP technique, namely, isotope labeling and non-radioisotope labeling. In this study we use the non-radioisotope labeling method. However, the sensitivity of silver nitrate staining method was similar to the radioisotope labeling method.[4] The front band (36 bp) showed on the polyacrylamide gel electrophoresis was PCR products of the internal standard following the characteristic oligonucleotide ladders started from 50 bp with 6 bp increments. The positive detection was defined when the number of the ladder bands greater than 4 (data is not shown). In this study, the quantity of protein applied on each assay equivalent to 10 to 20 tumor cell extracts. Table 1 shows the results of the telomerase expression in the human tumor and tumor-surrounding cells. Thirty-six out of 40 tumor samples expressed the telomerase activity, and 1 out of 39 tumor-surrounding tissues was positive. Thus, the sensitivity of the detection was a/(a+b)=90%, and the specificity of the telomerase to tumor cells was esti-mated as d/(d+b)=97%. The pathological data of the tested tumor patients are shown in Table 2. Specially, the telomerase activity exhibited in the tumor tissues did not correlate with the age, sex, and the stage of the tumor.

Table 1. Telomerase expression in gastrointestinal tumor tissues

Telomerase detection	Cases examined		
	Tumor tissue	Tumor-surrounding tissues	Total
Positive	36 (a)	1 (b)	37 (a+b)
Negative	4 (c)	38 (d)	42 (c+d)
Total (cases)	40	39	79

DISCUSSION

The length of telomere has been considered as the biological clock for the mitosis. It has been suggested that telomerase shortening is a control mechanism for cell life-span and proliferation in normal somatic cells. To maintain the telomere length is associated with telomerase activity. In human cells, the activity of telomerase exist only in rapid proliferating cells, such as embryo, bone marrow and germ cells. In most of differentiated normal somatic cells, the telomerase activity is hardly detected.[5] The study of high telomerase activity in tumor cells leads the hypotheses that activation of telomerase is necessary for tumor cells to achieve immortality. Shay[6] summarized the data from 196 normal samples, 410 distinct tumors, 2,031 malignant tumor samples and 690 tumor-surrounding tissues, and found that the percentage of detectable telomerase activity was 0.5%, 30%, 85% and 11%, respectively. Our data show the percentage of detectable enzyme activity was 90% in 40 tumor tissues and 2.6% in tumor-surrounding tissues, agreed with the results published by Shay et al. The finding that the telomerase activity was not associated with the stages of tumor progressing was also confirmed by this study. However, other mechanisms of telomere maintenance might be existed due to the finding by Bryan[7] whose results showed that among 35 immortalized cell lines, 15 had no detectable telomerase. The 15 telomerase-negative immortalized cell lines all had very long and heterogeneous telomeres of up to 50 kb, suggesting that the presence of lengthened telomeres is necessary for immortalization, and the maintaining of the stabilized telomeres can be achieved by the activation of telomerase or by an unidentified mechanism. Since most normal somatic

Table 2. The clinicopathologic data of 40 gastrointestinal tumor patients

	Telomerase activity	
	Positive	Negative
Age (yrs) range	37–63	26–77
Male:Female	3:1	23:13
Colorectal cancer (Dukes)		
A	0	11
B	1	7
C	1	8
D	1	1
Gastric cancer early stages		
I. Bulgy	0	2
II. Shallow	0	3
III. Depressed	0	1
Progressive stages		
I. Lump	0	1
II. Ulcerous	1	2
III. Widespread	0	0
Differentiation		
Well	0	4
Moderate	2	28
Poor	2	4

cells are telomerase-negative, the question that at which stage of the tumorogenesis telomerase is developed is of interested. It seems to be true that the human telomerase activity is greater in the late stage of tumor development than the early stages since the percentage of detectable telomerase is much greater in the tissues of patients at later stage of cancer than at early stage. Thus, to use telomerase activity as a biochemical marker for early tumor detection needs to be further studied. However, to apply this highly sensitive telomerase activity assay on a non-invasive diagnosis by detection of exfoliated cancer cell in urine[8] and in exfoliated cancer cells in colonic luminal washings[9] had already been investigated.

REFERENCES

[1] Morin GB. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell 1989; 59:521.

[2] Counter CM, Hirte HW, Bacchetti S, et al. Telomerase activity in human ovarian carcinoma. Proc Natl Acad Sci (USA) 1994; 91:2900.

[3] Kim NW, Piatyszek MA, Prowse KR, et al. Specific association of human telomerase activity with immortal cells and cancer. Science 1994; 266: 2011.

[4] Piatyszek MA, Kim NW, Weinrich SL, et al. Detection of telomerase activity in human cells and tumors by a telomeric repeat amplification protocol (TRAP). Methods Cell Sci 1995;17:1.

[5] Lundblad V, Wright WE. Telomeres and telomerase: a simple picture becomes complex. Cell 1996; 87: 369.

[6] Shay JW, Gazdar AF. Telomerase in the early detection of cancer. J Clin Pathol 1997; 50:106.

[7] Bryan TM, Englezou A, Gupta J, et al. Telomere elongation in immortal human cells without detectable telomerase activity. EMBO J 1995; 14: 4240.

[8] Yoshida K Sugino T, Tahara H, et al. Telomerase activity in bladder carcinoma and its implication for noninvasive diagnosis by detection of exfoliated cancer cells in urine. Cancer 1997; 79:362.

[9] Yoshida K, Sugino T, Goodison S, et al. Detection of telomerase activity in exfoliated cancer cells in colonic luminal washings and its related clinical implications. Br J Cancer 1997; 75:548.