Alteration of Telomere Length in Gastric Carcinoma

Shuman Liu[1], Jie Fang[2], Wei Zhang[1], Qinxian Zhang[1]

1. Department of Histology and Embryology, Basic Medical College, Zhengzhou University, Zhengzhou, Henan 450052, China

2. Department of Internal Medicine, Huaxian People's Hospital, Huaxian, Henan 456400, China

Abstract: Objective. To evaluate the alteration of telomere length in gastric carcinoma. **Methods.** Southern blot was used to detect the telomere length in gastric carcinoma, matched adjacent tumor tissue and normal gastric mucosa. **Results.** In 32 samples, the telomere length in gastric carcinoma, matched adjacent tumor tissue and corresponding normal gastric mucosa were 5.088 ± 1.712 kb, 5.969 ± 1.659 kb and 6.728 ± 1.707 kb respectively. There was no significant correlation between the telomere shortening in gastric carcinoma and the pathological grades, invasion depth, lymph node metastasis and tumor size. **Conclusion.** Telomere length in gastric carcinoma shortened obviously than that in corresponding normal gastric mucosa and adjacent tumor tissue. Telomere shortening may not be used as a sensitive biomarker to judge the malignance of gastric carcinoma. [Life Science Journal. 2006;3(3):25-28] (ISSN: 1097-8135).

Keywords: gastric carcinoma; telomere length; Southern blot

Abbreviations: BCIP: 5-bromo-4-chloro-3-inddylphosphate; DIG: digoxin; NBT: nitro-blue tetrazolium; TRF: terminal restriction fragments; SSC: sodium saline citrate

1 Introduction

Telomeres are unique structures at the physical ends of linear eukaryotic chromosomes. In most eukaryotes, telomeric DNA consists of simple repetitive sequences with G-rich 3′ terminal. In human somatic cells, telomeres have 500 - 3000 repeats of TTAGGG, which gradually shorten with age *in vivo* and *in vitro*[1]. It has been reported that telomere shortening occurs in a subset of tumors[2], but the alteration of telomere length in gastric carcinoma remained to be elucidated. About this there are different opinions[3,4]. In this study, we examined the telomere length in gastric carcinoma, matched adjacent tumor tissue and corresponding normal gastric mucosa by using analysis of terminal restriction fragments (TRF), with special reference to their clinical features and histological findings. From the accumulated data, we determined whether the telomere length is associated with gastric carcinogenesis and the development of gastric carcinoma.

2 Materials and Methods

2.1 Materials

Thirty-two samples from gastric carcinoma, with matched adjacent tumor tissue and corresponding normal gastric mucosa, were studied. In each case, tumor tissue, matched adjacent tumor tissue and corresponding normal mucosa, at least 5 cm apart, were obtained from surgically dissected stomach. The patients never received radiotherapy and chemotherapy, including twelve males and twenty females. The age of the patients varied in the range of 25 - 69 years old. All tissues were frozen in liquid nitrogen. Considering the morphological characteristics, all of the samples from the gastric carcinoma were identified as ulcerating, papillary and infiltrating carcinoma. Histological examination revealed 14 cases of well differentiated adenocarcinoma, 16 cases of poorly differentiated adenocarcinoma and 2 cases of mucous signet-ring cell adenocarcinoma.

2.2 Genomic DNA isolation and Southern blot

Took out the fresh frozen samples from liquid nitrogen and rapidly ground into powder. High-molecula-weight DNA was prepared from each sample by digestion with proteinase K and extraction with phenol/chloroform. Deposited and condensed genomic DNA with ethanol. Identified DNA purity and concentration with ultraviolet spectrophotometer(HITACHI). 1% agarose gel electrophoresis revealed the genomic DNA integrity. Equivalent amounts of tumor and constitutional DNA (3 μg) were digested overnight at 37 ℃ with 5 μl *Hinf* Ⅰ (TaKaRa, Kyoto, Japan). Thus, the terminal restriction fragments (TRFs), containing both the subtelomeric repetitive DNA and telomeric 5′-TTAGGG-3′ repeats, were liberated. The TRF de-

termining telomeric length were separated by 0.6% agarose gel electrophoresis, denatured and neutralized, and then transferred by capillary transfer onto positive nylon membranes (Osmonics) for Southern blotting. The filters were prehybridized in a hybridization buffer (Sangon) for 6 h at 42 ℃, then hybridized with telomere probe 5′-$(CCCTAA)_3$-3′ labeled with digoxin (DIG) random labeling and detection kit (Boshide, Wuhan, China) in hybridization buffer overnight at 42 ℃. The filters were washed twice in 2 × sodium saline citrate (SSC)/0.1% sodium dodecyl sulfate (SDS) for 5 min at room temperature and then washed twice in 0.2 × SSC/0.1% SDS for 10 min at 58 ℃. The filters were blocked for 1 h at room temperature with blocking buffer (Pierce) and incubated for 1 h at 37 ℃ in a Anti-Dig-Ap mixture that had been diluted 1∶2500 in the blocking buffer. The filters were washed twice in washing buffer for 30 min. Then incubated for 2 min at room temperature in coloring buffer. After mixing with NBT and BCIP (2∶1, Promega), the filters were kept still in shaded corner for color development until the ribbon appeared.

2.3 Densitometry and mean telomere length measurements

The telomeric lengths were quantified by densitometric analysis of the ribbon using Gel imaging analysis system(Gene Genius). The mean telomere length in each sample was calculated as reported[5].

2.4 Statistical analysis

The analysis were conducted with SPSS 10.0 statistical software. Results were expressed as $\bar{x} \pm s$. χ^2 tests, t and K-W tests were used, and a P value < 0.05 was set statistically significant.

3 Results

3.1 Mean telomere length in gastric carcinoma, matched adjacent tumor tissue and normal gastric mucosa

In 32 samples, the telomere length in normal gastric mucosa varied in the range of 4.0 kb − 11.0 kb, the mean telomere length was 6.728 ± 1.707 kb. The telomere length in matched adjacent tumor tissue varied in the range of 3.0 kb − 10.0 kb, the mean telomere length was 5.969 ± 1.659 kb. The telomere length in gastric carcinoma varied in the range of 2.0 kb − 9.0 kb, the mean telomere length was 5.088 ± 1.712 kb. There were significantly statistical differences between three groups ($F = 7.529$, $P = 0.01$). The mean telomere length in normal gastric mucosa, matched adjacent tumor tissue and gastric carcinoma shortened obviously in turn(Table 1, Figure 1). Otherwise, in 3 cases of gastric carcinoma, the mean telomere length slightly shortened, and was even a little longer than that of the normal mucosa(Figure 2).

Table 1. The mean telomere length in gastric carcinoma, adjacent tumor tissues and normal gastric mucosa

Lesions	TRF(kb, $\bar{x} \pm s$)	Significance
Carcinoma	5.088 ± 1.712	
Adjacent tumor tissues	5.969 ± 1.659	$P = 0.01$
Normal tissues	6.728 ± 1.707	

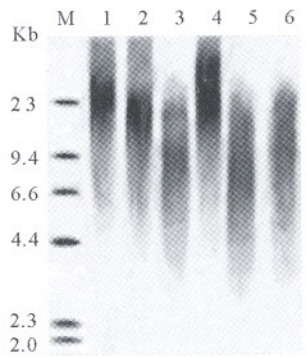

Figure 1. Southern blotting
M: Dig MW marker; Lane 1 and Lane 4: matched normal gastric mucosa; Lanes 2 and 5: adjacent tumor tissue; Lane 3 and Lane 6: gastric carcinoma

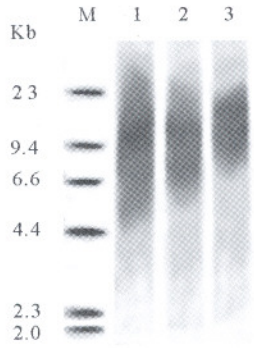

Figure 2. Southern blotting
M: Dig MW marker; Lane 1: matched normal gastric mucosa; Lane 2: adjacent tumor tissue; Lanes 3: gastric carcinoma

3.2 Correlation between telomere length shortening and clinical factors in gastric carcinoma

Several variables, such as age, sex, tumor size, histology, infiltrating depth and tumor stage with lymph node metastasis, were examined for potential links with telomere shortening in the gastric carcinoma group. Age was significantly associated with telomere shortening in normal gastric mucosa. While the mean telomere length showed shortening tendency with age in carcinoma group

and adjacent tumor group, obvious statistical significance was absent (Table 2). Similarly, tumor size, histology, infiltrating depth, tumor stage with lymph node metastasis and sex were not significantly correlated with telomere shortening in gastric carcinoma($P>0.05$).

Table 2. Mean telomere length in different age group

Age(year)	Cases(n)	TRF(kb, $\bar{x}\pm s$)		
		Normal tissue	Adjacent tumor tissue	Carcinoma
20－29	3	10.767±0.252	9.533±0.643	8.476±0.503
30－39	7	7.471±0.214	6.900±0.252	6.229±0.263
40－49	10	6.780±0.235	5.980±0.308	5.010±0.538
50－59	4	6.275±0.457	5.650±0.311	4.825±0.624
60－69	8	4.725±0.656	3.936±0.940	3.050±1.149
P		$P<0.05$	$P>0.05$	$P>0.05$

4 Discussion

A telomere is a group of tandem-repeat DNA sequences located at the ends of eukaryotic chromosomes. Telomere is thought to stabilize chromosomes and protect them from end-to-end fusion or exonucleolytic degradation[1].

Telomere has close relationship with tumorigenesis. Telomeres cannot be replicated completely by DNA polymerases because the enzymes cannot accomplish the coping processes to the very end of DNA strands. Therefore, the length of telomere decreases gradually with the increasing number of cell divisions and therefore with aging, resulting in chromosome instability and genetic changes that may lead to tumor development[6]. Alterations of telomere length have been reported in a subset of tumors including colorectal carcinoma[7], hepatic carcinoma[8], skin base cell carcinoma and renal cell carcinoma[9,10], but they were not consistent, and even can examine longer telomere in carcinoma. Some papers revealed that telomere shortening was related to tumor size, histological type, infiltrating depth, lymph node metastasis[11,12]. Kondo[13] believed that telomere shortened progressively in gastric carcinoma with development of tumor.

Our current finding that the mean telomere length shortened in the order of normal gastric mucosa, matched adjacent tumor tissue and gastric carcinoma may also suggest reduction of telomere occurs in early period of gastric carcinogenesis, causes chromosome instability and accelerates development of gastric carcinoma. This is consistent with theory set by Meeker[14]. Another finding of the current study is that in 3 cases of gastric carcinoma, the mean telomere length slightly shortened, and was even a little longer than that of the normal mucosa. There are several possible reasons about this: telomerase activation was expressed at the early stage of tumorigenesis and compensate shortened telomere; stroma cells are abundant in tumor tissues, and their DNA may affect analysis of TRFs; telomere prolonged mechanism beyond telomerase activation exists. Our current study also indicated that telomere shortening in gastric carcinoma was not related to clinical pathological parameters.

From all the above, though reduction of telomere is one early molecular event of gastric carcinogenesis, the mean telomere length in gastric carcinoma is also decided by other factors and lack of significant correlation with clinical pathological parameters. We deduce that telomere shortening may not be used as a sensitive biomarker to diagnose gastric carcinoma and judge the malignant degree.

Correspondence to:
Shuman Liu
Department of Histology and Embryology
Basic Medical College
Zhengzhou University
Zhengzhou, Henan 450052, China
Telephone: 86-137-8362-1327
Email: donna79320@126.com

References

1. Blackburn. Structure and function of telomeres. Nature 1991; 350(3):569－73.
2. Meeker AK, Argani P. Telomere shortening occurs early during breast tumorigenesis: a cause of chromosome destabilization underlying malignant transformation? J Mammary Gland Biol Neoplasia 2004; 9(3):285－96.
3. Furugori E, Hirayama R, Nakamura KI, *et al*. Telomere shortening in gastric carcinoma with aging despite telomerase activation. Cancer Res Clin Oncol 2000; 126(8):481－5.
4. Maruyama Y, Hanai H, Kaneko E. Telomere length and telomerase activity in intestinal metaplasia, adenoma and

well differentiated adenocarcinoma of the stomach. Nippon Rinsho 1998; 56(5): 1186 - 9.
5. Hastie ND, Dempster M, Dunlop MG, *et al*. Telomere reduction in human colorectal carcinoma and with aging. Nature 1990; 346(6287):866 - 8.
6. Shay JW. Molecular pathogenesis of aging and cancer: are telomeres and telomerase the connection? J Clin Pathol 1997; 50(10):799 - 800.
7. Kim HR, Kim YJ, Kim HJ, *et al*. Telomere length changes in colorectal cancers and polyps. Korean Med Sci 2002; 17(3):360 - 5.
8. Yokota T, Suda T, Igarashi M, *et al*. Telomere length variation and maintenance in hepatocarcinogenesis. Cancer 2003; 98(1): 110 - 8.
9. Wainwright J, Middleton PG, Rees JL. Changes in mean telomere length in basal cell carcinomas of the skin. Genes Chromosome Cancer 1995; 12(6):45 - 9.
10. Dahse R, Fiedler W, Ernst G, *et al*. Changes in telomere lengths in renal cell carcinomas. Cell Mol Biol 1996; 42(9): 477 - 85.
11. Gordon KE, Ireland H, Roberts M, *et al*. High levels of telomere dysfunction bestow a selective disadvantage during the progression of human oral squamous cell carcinoma. Cancer Res 2003; 63(2): 458 - 67.
12. Frazil PA, Glickman J, Jiang S, *et al*. Differential impact of telomere dysfunction on initiation and progression of hepatocellular carcinoma. Cancer Res 2003; 63(16): 5021 - 7.
13. Kondo T, Oue N, Yoshida K, *et al*. Expression of POT1 is associated with tumor stage and telomere length in gastric carcinoma. Cancer Res 2004; 64(2): 523 - 9.
14. Meeker AK, De Marzo AM. Recent advances in telomere biology: implications for human cancer. Curr Opin Oncol 2004; 16(1): 32 - 8.

Received June 10, 2006