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Telomere Length Changes in Colorectal Cancers and Polyps

Telomere shortening and telomerase activation occur frequently in cases of colorectal carcinoma. In this study, we correlated the clinicopathological parameters with the telomere length in colorectal carcinomas, colonic polyps, and normal colonic tissues. We also investigated whether the telomere length changes reflect the biologic behavior of tumors and different modes of tumor development. Telomere length was determined by terminal restriction fragment Southern blot analysis in 20 invasive colorectal carcinomas and normal mucosa from the same patients. We also examined 20 colonic polyps and associated normal mucosa. Telomere shortening was detected in 16/20 (80%), and telomere elongation in 2/20 (10%) cases of colorectal carcinoma, and no changes in 2 subjects. In the colonic polyp patients, shortening was detected in 4/20 (20%), elongation in 6/20 (30%), and no change in 10/20 (50%). The frequency of telomere shortening was significantly different between colorectal carcinoma and polyp groups. Decreased telomere length was noted in 92.9% (13/14) of Dukes' C and 50% (3/6) of Dukes' B. The difference between these two sub-groups was statistically significant. This study suggests that the telomere length in colorectal carcinomas is decreased upon the development of malignancy. A significant difference in telomere length between polyps and invasive colorectal carcinomas may reflect a different biologic behavior of colorectal carcinomas.

Key Words : *Telomere; Colorectal Neoplasms; Colonic Polyps; Clinicopathological Characteristics*

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INTRODUCTION

A telomere is a group of tandemly repeated DNA sequences located at the ends of eukaryotic chromosomes (1). In humans, telomeres are composed of 800-3,000 repeats of 5′-TTA GGG-3′, constituting 5-15 kilobases in total (2, 3). Telomeres are thought to stabilize chromosomes and protect them from end-to-end fusion or exonucleolytic degradation (4-7). Telomeres cannot be replicated completely by DNA polymerases because the enzymes cannot continue copying to the very ends of the DNA strands. Therefore, the telomere length gradually decreases with successive cell divisions (and thus with aging), resulting in chromosomal instability and genetic changes that may lead to tumor development (5, 8, 9). A reduction in telomere length has been reported in a subset of tumors, including colorectal and renal cell carcinomas and childhood leukemia (9-11). Telomerase is a ribonucleoprotein, the RNA component of which acts as a template for the synthesis of the telomere sequence by reverse transcription. Avoiding telomeric shortening by expressing telomerase may contribute to an immortality phenotype (4).

The variations in telomere length between individual colorectal carcinomas may represent the different stages of carcinogenesis and therefore, the biologic behavior of individual tumors. It has been reported that telomere shortening frequently occurs in both colorectal carcinomas and colonic adenomas, but little telomerase activation has been noted in colonic adenomas, whereas it is already strongly activated in early colorectal carcinoma (9, 12).

In this study, we examined the telomere lengths in colorectal carcinomas, matched adjacent normal colon tissues, and polyps, with special reference to their clinical features and histological findings. From the accumulated data, we determined whether the telomere length is predictive of the biologic characteristics of human colorectal carcinomas.

MATERIALS AND METHODS

Materials

Twenty samples from invasive colorectal carcinomas, with matched adjacent normal colorectal tissues, and twenty samples from colonic polyps, with adjancent normal colorectal tissues, were studied. In each case, tumorous and normal mucosa samples, at least 5 cm apart, were obtained from

surgically resected colon or rectum. There was a patient who had a carcinoma and a polyp in the same specimen. These potentially curative procedures, i.e., surgical resections for colorectal cancer and colonoscopic or sigmoidoscopic polypectomy for colorectal polyps, were performed between January and October 2000. The embedded tissues were frozen in liquid nitrogen. In the colorectal carcinoma group, there were 8 cases of Dukes' B and 12 cases of Dukes' C disease. Histological examinations revealed 14 cases of well differentiated adenocarcinoma and 6 cases of moderately differentiated adenocarcinoma. All of the samples from the colorectal polyp group were identified as tubular adenoma, except for one case of tubulovillous adenoma.

DNA Isolation and Southern Blot Analysis

High-molecular-weight DNA was prepared from each sample by digestion with proteinase K and extraction with phenol/chloroform. Equivalent amounts of tumor and constitutional DNA (10 μg) were digested overnight at 37℃ with 10 units/μg DNA each of *RsaI/HinfI* (TaKaRa, Kyoto, Japan). Thus, the terminal restriction fragments (TRFs), containing both the subtelomeric repetitive DNA and telomeric 5′-TTAGGG-3′ repeats, were liberated. The TRFs determining telomeric length were separated by agarose (0.7%) gel electrophoresis, denatured with 0.5 M NaOH and 1.5 M NaCl, and then transferred by capillary transfer onto nylon membranes (Hybond -N; Amersham International plc, Amersham, U.K.) for Southern blotting. The filters were prehybridized in a hybridization buffer (TeloQuant; PharMingen, San Diego, CA, U.S.A.) for 1 hr at 65℃ and then hybridized with a biotinylated telomere probe $(TTAGGG)_4$ (TeloQuant) in hybridization buffer overnight at 65℃. 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 15 min at 42℃. The filters were blocked for 1 hr at room temperature using blocking buffer (TeloQuant) and shaken for 1 hr at room temperature in a streptavidin-horseradish peroxidase (<25 ng/mL) mixture that had been diluted in the blocking buffer. The filters were washed four times in 0.1% Tween-20 (Boehringer Mannheim GmbH, Mannheim, Germany) in PBS for 10 min. After mixing with equal volumes of stable peroxide and luminol/enhancer (TeloQuant), the hybridized probe was shaken for 5 min and chemiluminescence was detected according to the manufacturer's instructions (TeloQuant). The filters were exposed on radiography film (T-MAT; Kodak, U.S.A.) for 30-60sec.

Densitometry and Mean Telomere Length Measurements

The telomeric lengths were quantified by densitometric analysis of autoradiographs using a transmitter scanning videodensitometer (Zenith Video Densitometer; Biomed, Fullerton, CA, U.S.A.). The mean telomere length in each sample was then identified as the peak intensity of the telomere length (in kb) by densitometry. We suspected telomere length change as shortened when the mean TRF length was below 80% of that of the normal mucosa and lengthened when it was above 120% of normal mucosa.

Statistical Analysis

The χ^2 test (SPSS 10.0) was used for the telomere length comparisons between groups.

RESULTS

Mean telomere length in colorectal carcinomas and polyps

The mean telomere length in the colorectal carcinoma group (n=20; 7.12±0.14 kb) was significantly shorter than that of the normal colonic mucosa (n=20; 9.25±0.16 kb). The mean telomere length in colonic polyps (n=20; 9.41±0.53 kb) was slightly longer than that of the normal mucosa.

Telomere shortening in colorectal carcinomas and polyps

Colorectal carcinomas showed shortened (16/20; 80%), elongated (2/20; 10%), and unchanged (2/20; 10%) mean telomere lengths (Fig. 1). In the colonic polyp group, 4/20 (20%) showed a telomere shortening, 6/20 showed elongation, and the remainder (10/20) showed no changes in telomere length (Fig. 2). The frequency of telomere shortening was significantly different between the colorectal carcinoma and polyp groups (p<0.05)(Table 1). Among those polyps, which

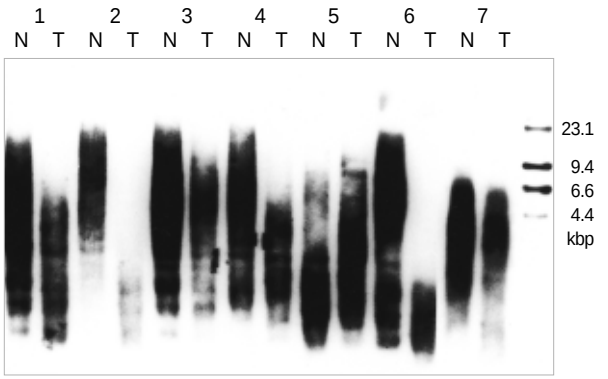

Fig. 1. Terminal restriction fragment (TRF) analysis of colorectal carcinomas. All tumor tissues, except one (Case 5), show a decrease in the mean TRF length. N: constitutional DNA of normal mucosa; T: tumor tissue; *: increase in the mean TRF length; kbp: kilobase pairs.

were identified by histopathologic findings, showed 14 tubular adenomas with mild dysplsia, 5 tubular adenomas with moderately dysplasia and one tubulovillous adenoma with mild dysplasia. Among 14 tubular adenomas with mild dysplasia, telomere shortening was showed in 3 cases (21.4%) and that of tubular adenoma with moderately dysplasia showed 1 case (20%). The tubulovillous adenoma showed unchanged telomere length. There was no significant difference among histopathologic classification of polyps (Table 2). One case, containing both a carcinoma and a polyp, showed a telomere shortening in the carcinoma and telomere elongation in the polyp (Fig. 3).

Relationship between telomere length shortening and clinical factors in colorectal carcinoma

Several variables, such as age, sex, tumor location, histology, tumor size, gross findings with Borrmann classification, and tumor stage with lymph node metastasis, were examined for potential links with telomere shortening in the colorectal carcinoma group. Age and sex were not significantly associated with telomere shortening in colorectal carcinoma (Table 3). Tumor location, tumor histology, tumor size, and gross findings with Borrmann classification were not significantly linked with telomere shortening. Indeed, the only factor that correlated with telomere shortening in a statistically significant manner in colorectal carcinoma was tumor stage with lymph node metastasis (Table 4). A decreased telomere length was noted in 92.9% (13/14) of Dukes' C and 50% (3/6) of Dukes' B. The difference between these two sub-groups was statistically significant ($p<0.05$).

Table 1. Telomere shortening in colorectal carcinomas and polyps

Lesions	No.	Telomere length change Shortened /elongated /unchanged	% of decrease	Signficance
Carcinoma	20	16/2/2	80	p=0.001
Polyp	20	4/6/10	20	

DISCUSSION

Telomeres, the nucleoprotein complexes at the ends of eukaryotic chromosomes, consist of tandem arrays of 5′-TTAGGG-3′ repeats associated with specific proteins that stabilize the chromosome ends and protect against enzymatic end-degradation (1, 13, 14). Telomere length is maintained by balancing processes that lengthen and shorten telomeres (15). Since the telomeric loss is due to an end-replication defect during progressive cell divisions, telomere shortening is regarded as a molecular clock that counts the number of cell divisions and determines the onset of cellular senescence (5, 16, 17). Thus, the telomere length is an indicator of replicative history and may also determine the replicative potential of cells (5, 17, 18). In normal somatic cells, telomere progresses at a rate of 50-100 bp per cell division and eventually leads to growth arrest. Critically shortened telomeres, when cells

Table 2. Telomere length changes in colorectal polyps according to histopathologic classification

Histolopatholgic classification	No.	Telomere length change Shortened/elongated/unchanged	% of decrease	Signficance
Tubular adenoma with mild dysplasia	14	3/5/6	21.4	
Tubular adenoma with moderately dysplasia	5	1/2/2	20	p>0.05
Tubulovillous adenoma with mild dysplasia	1	0/0/1	0	

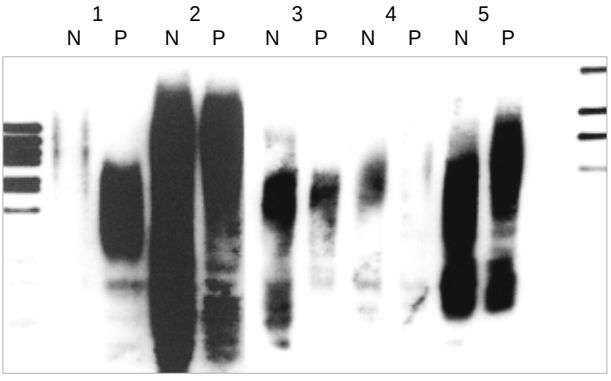

Fig. 2. Terminal restriction fragment (TRF) analysis of colonic polyps. Cases 1 and 2 show a decreased telomere length, Case 3 shows no change and remaining cases show an increase in the mean TRF length. N: normal mucosa; P: polyp.

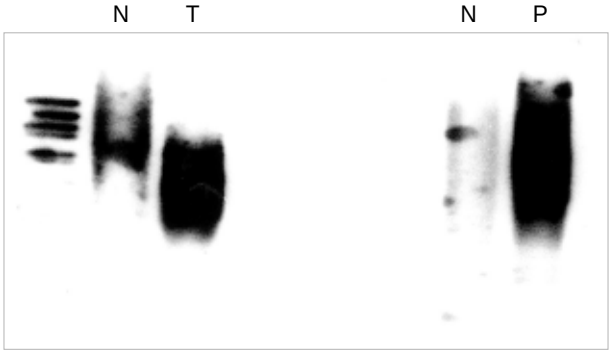

Fig. 3. Terminal restriction fragment (TRF) analysis of a patient with both carcinoma and a polyp in the same specimen. The mean TRF length is decreased in the tumor tissue and increased in the polyp. N: normal mucosa; T: tumor; P: polyp.

Table 3. Correlation between telomere length and clinical variables in colorectal cancer

	No.	Shortened telomere	Elongated telomere	Unchanged telomere	Significance
Sex					NS*
Male	12	10	1	1	
Female	8	6	1	1	
Age (yr)					NS
40-49	1	1	0	0	
50-59	5	5	0	0	
60-69	8	7	1	0	
70-79	6	3	1	2\	

*not significant

Table 4. Correlation between telomere length and clinical variables in colorectal cancer

	No.	Shortened telomere	Elongated telomere	Unchanged telomere	Significance
Tumor location					NS
Ascending colon	3	2	0	1	
Transverse colon	2	1	1	0	
Descending colon	3	2	1	0	
Sigmoid colon	5	5	0	0	
Rectum	7	6	0	1	
Tumor histology					NS
Well differentiated	14	12	2	0	
Moderately differentiated	6	4	0	2	
Tumor size					NS*
< 3.0 cm	6	3	2	1	
3.1 - 4.9 cm	5	4	0	1	
> 5.0 cm	9	9	0	0	
Borrmann type					NS
I	3	3	0	0	
II	11	9	1	1	
III	6	4	1	1	
IV	0	0	0	0	
Stage					$p<0.05$
B	6	3	1	2	
C	14	13	1	0	

*not significant

are no longer able to protect the ends of chromosomes, cause chromosome fusion and massive genomic instability that may contribute to age-related clonal disorders (19-21).

In malignant cells, measurements of telomere length have been used as markers of cell proliferation and population doubling (1, 4, 8-10, 17, 22-27). Since the acquisition of mean TRF values alone may be inaccurate, the mean and peak values were analyzed in this study. Both values were lower in the majority of the colorectal carcinomas when compared to the normal adjacent tissues. Similarly, adenoma telomeres have previously been reported to be shorter than those in adjacent mucosal tissues (9). In contrast, we found that telomere lengths in polyps varied, i.e., they were shorter than, or similar in length to the telomeres in normal colonic tissues.

Telomerase activity did not correlate significantly with telomere length in colorectal carcinomas (28). This finding suggests a mechanism for telomere maintenance whereby telomerase potency is balanced with the speed of telomere shortening. It is also worth noting that factors other than telomerase might participate in maintaining the telomere length (28). Interestingly, the telomere length in primitive hematopoietic stem cells decreases despite telomerase activity (29). However, it has been reported that some human tumors and some tumor-derived cell lines display telomere elongation in the apparent absence of telomerase activity (27, 30). Furthermore, we did not investigate these factors in our study, alternative mechanisms for telomere elongation that are independent of human telomerase RNA have been demonstrated in telomerase-negative, immortal human cells (31). Recently, protein factors, such as TRF 1, TRF 2, and Pin 2 that bind to repeat DNA sequences in telomeres, have been discovered (32-34). These proteins are thought to affect telomere length in human cell lines in vitro by a mechanism that remains to be elucidated.

In most previous studies, telomere shortening was observed in colorectal carcinomas, although there were variations in the extent of shortening. In addition, some colorectal carcinomas possessed longer telomeres than corresponding normal colonic tissues. Because of these inconsistencies, it was important to clarify the significance of telomere length in human colorectal carcinomas. Therefore, we hypothesized that the telomere length might reflect the current biological features of individual tumors that had been imprinted during carcinogenesis and tumor development. Shimoda et al. classified colorectal carcinomas into 2 types according to their growth patterns: polypoid and nonpolypoid growth carcinomas (35). According to this classification, nonulcerating polypoid carcinomas are regarded as polypoid growth carcinomas that arose from adenoma. We anticipated that colorectal carcinomas derived from adenomas might have shorter telomeres. Takagi et al. showed that telomere shortening in colorectal carcinomas occurred more frequently in nonulcerating polypoid carcinomas than in ulcerating carcinomas (28). They also reported that the frequency of telomere shortening was higher in ascending colon carcinomas than in sigmoid colon and rectal carcinomas. In our study, there were no significant differences in gross tumor patterns and tumor locations. There is considerable evidence to indicate that proximal and distal colon carcinomas may have different molecular characteristics, such as DNA content, karyotype, oncogene expression, allelic deletions, DNA replication errors (RER), loss of heterozygosity (LOH), and microsatellite instability (MSI) (36-43). We did not investigate these factors in our study. Takagi et al. also reported that tumor histology, clinical stage, and the presence of metastasis in colorectal carcinomas did not correlate with the differences in telomere length. In our study, Dukes' stage with lymph node metastasis was a significant factor in determining the telomere length in colorectal carcinomas. Engel-

hardt et al. reported that telomeres were longer in tumors with late-stage colorectal carcinoma, than in early-stage tumors (44). These discrepancies might be due to racial differences in the subject populations or the small number of cases studied.

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