Telomere Length and Telomerase Activity in Extra-abdominal Desmoid Tumors

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Abstract. *The telomere biology of extra-abdominal desmoids was investigated. In 12 specimens, telomere length was assessed by Southern-blot analysis and telomerase activity was measured using a PCR-based TRAP assay. There was a significant correlation between telomere length and tumor size, with telomeres being shorter with increasing tumor size ($p=0.049$), and between telomere length and PCNA-positive cell rate, with telomere shortening with increased positive cell rate ($p=0.017$). There was a significant correlation between telomerase activity and age at surgery, with increased activity with younger age ($p=0.015$). Telomere length increased with recurrence, but telomerase activity decreased, and rate of PCNA-positive cells became lower, whenever the tumors were recurrent. Decreasing telomere length correlated with tumor size, probably due to increased duration of proliferation in the tumor, and tumor aggressiveness. Recurrent case results may be due to a lower rate of cell division and the presence of telomerase activity.*

An extra-abdominal desmoid tumor is a rare neoplasia of fibroblastic origin, composed of fibroblast-like cells and abundant collagen that may arise within musculoaponeurotic tissue. This tumor appears histologically in benign and manifests no malignant transformation or metastasis. However, it is locally aggressive, invasive, and expresses permeative clinical behavior. Interestingly, self-limited oncological features have also been reported (1, 2). Several papers investigating the alteration of telomere length in tumors with a high malignancy have been reported (3-5). Telomerase is a reverse transcriptase that contributes to the maintenance of the telomere repeat sequence, and, therefore, enables cellular immortality (6, 7). Accordingly, it has been found that almost all cancer cell lines express activated telomerase (5, 8-10). Despite such findings on telomeres and telomerase activity, there are few reports that show an association between the levels of telomerase activity or telomere length and patient prognosis in extra-abdominal desmoid. The aim of our study was to investigate whether there is a correlation between telomerase activity levels or telomere length and other clinical features of desmoids, for the possible use of these factors as parameters of aggressiveness. Cell proliferation was investigated using immunohistochemical staining for proliferation cell nuclear antigen (PCNA).

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Key Words: Extra-abdominal desmoid tumor, telomere length, telomerase activity, proliferation cell nuclear antigen (PCNA) staining.

Materials and Methods

Clinical data of patients. Nine patients with primary tumors underwent surgical treatment for extra-abdominal desmoid tumor at Hiroshima University Hospital, between 1992 and 2002 (Table I). There were 4 males and 5 females, with an average age at diagnosis of 36.4 years (range, 14 to 66 years). Tumors were located in the lower leg (3 patients), thigh (3), chest wall (1), lumbar (1) and back (1). Informed consent was obtained from patients before surgery. For all patients, marginal or wide resection was performed with no adjuvant therapy. Tumor size was evaluated by measurement of the largest diameter on MR images at diagnosis. Average tumor size was 8.5 cm (range 3.0 to 13.0). The average duration of postoperative observation was 70.1 months (range: 34 to 121 months). After surgery, seven patients were continuously disease-free and two patients showed local recurrence, of whom one experienced recurrence once, and the other experienced recurrence twice.

Tissue samples. One sample was collected by resection from each primary tumor and each recurrent tumor. Therefore, a total of 12 tumors samples were obtained from the patients. Normal muscle samples were collected from all patients for use as control. All tissues were immediately frozen and stored at –80°C until use.

Table I. *Data of 12 desmoid tumors.*

Case (Patient)	Gender	Age (yr)	Tumor location	Observation period (months)	Tumor size (cm)	Telomere length (kb)		Telomerase activity (TPG)	PCNA-positive cells (%)
						Normal muscle tissue	Tumor		
1 (A)	Female	14	lower leg	121	8	13.5	8.6	0.23	27.5
2 (B)	Male	37	chest wall	79	7	10.5	12.2	0.23	12.8
3 (C)	Male	17	lower leg	67	13	9.5	7.2	0.51	30.4
4 (D)	Female	66	lumbar	66	7.5	11	9.5	0	17.3
5 (E)	Male	58	back	34	8.5	8.5	8.5	0.19	20.9
6 (F)	Female	43	thigh	84	12	10.5	7.8	0.05	25.2
7 (F)	*	46	as above	34	4.5	10.8	9.2	0	16.8
8 (G)	Male	22	lower leg	55	9	10.9	8.5	0.34	38.1
9 (G)	**	24	as above	25	3	11	9.4	0.18	7.5
10 (G)	***	26	as above	52	8.5	10	10.7	0.06	5.2
11 (H)	Female	22	thigh	36	10	9	10	0.3	20.4
12 (I)	Female	49	thigh	89	11	9	9.2	0	15

*recurrent case of patient 6; **, ***recurrent cases of patient 8.

Telomere length analysis using Southern-blotting. Genomic DNA was isolated from frozen samples of desmoid tumor and paired normal muscles. For the analysis of telomere length, 2 μg of DNA was digested to completion with 10 units of Hinf1 (Cosmo Bio, Tokyo, Japan), electrophoresed on 0.8% agarose gels, and then blotted onto nitrocellulose filters. The filters were hybridized to a ^{32}P-labeled $(TTAGGG)_4$ probe (Takara, Otsu, Japan), washed and then autoradiographed, as previously reported (4). The mean length of TRFs was estimated at the peak position of hybridization signal using a Bioimage Analyzer (BAS 2000, FUJIFILM, Tokyo, Japan) and MacBass software (FUJIFILM).

Telomerase assay. Evaluation of telomerase activity was performed using TRAP (telomeric repeat amplification protocol) assay as previously described (7). The concentration of tumor protein was measured using the BCA Protein Assay Kit (Pierce Chemical Co., Rockford, IL, USA), and an aliquot containing 1ug of tumor protein was used for each TRAP assay. The levels of telomerase activity were measured using the TRAPeze XL Telomerase Detection Kit (Intergen Co., NY, USA), which is a semiquantitative fluorescein-labelled PCR system, with the use of a PCR internal control, and were expressed as total product generated (TPG) units.

PCNA immunohistochemistry. PCNA immunostaining was performed on a paraffin-embedded tumor section that was retrieved from the specimens. For staining, paraffin blocks were cut into 5-μm sections and placed onto slides, followed by deparaffinization in xylene, and then rehydration in alcohol and water. Three percent H_2O_2 was used to block endogenous peroxidase activity. After washing three times in PBS, nonspecific antibody binding was blocked by incubating sections in protein blocking solution (Dako Cytomation, Carpenteria, CA, USA). Sections were then incubated overnight with PCNA (Dako). Subsequently, they were incubated in the labeled streptavidin biotin polymer (Dako), followed by 0.05% 3,3'-diaminobenzidine (DAB) in PBS with H_2O_2 as a substrate. Finally, sections were lightly counterstained with hematoxylin. The potential for cell proliferation was calculated using the following equation: PCNA-positive cell number / total cell number x100 (%).

Statistical analysis. Correlations between groups (telomere length, telomerase activity level, and each of the other factors) were performed using paired and unpaired Student's *t*-test, or linear regression analysis by the least squares method, where appropriate. In order to adjust for multiple comparisons when one way ANOVA showed a significant difference between groups, the Fisher LSD *post-hoc* test was used to identify which group differences accounted for the significant *p*-value. Data are presented as the mean±SD. In all analyses, a *p*-value of <0.05 was considered to indicate significance. All statistical analyses were performed on a personal computer using the statistical package Statview, Version 5.0 (Abacus Concepts, Berkley, CA, USA).

Results

Telomere length and clinical results. The telomere length of the adjacent normal muscles ranged between 8.5 and 13.5 kb, whereas those of desmoid tumors varied between 7.2 and 12.2 kb (Table I). Telomere length was 9.2±1.3 kb in tumors, and 10.4±1.3 kb in normal tissues. There was no significant difference between telomere lengths of tumors and those of normal tissues ($p=0.065$). There was a significant correlation between telomere length of primary tumor (except recurrent tumors) and tumor size measured using MR Images ($r=0.669$, $p=0.049$) (Figure 1). There was a significant correlation between telomere length and PCNA stainig ($r=0.670$, $p=0.017$) (Figure 2). There was no significant correlation between telomere length and age at surgery ($r=0.079$, $p=0.808$) on gender ($p=0.654$).

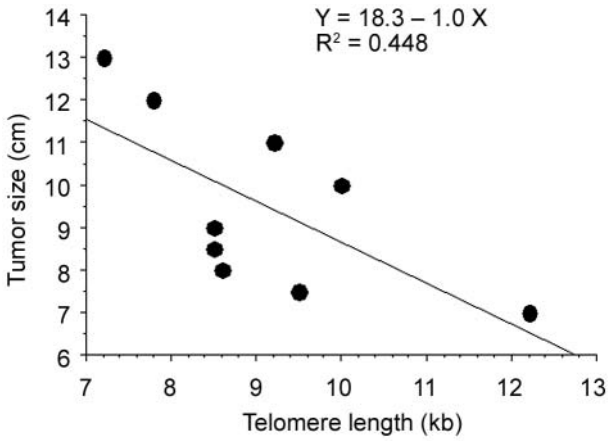

Figure 1. *Correlation between telomere length of tumor and tumor size as measured with MRI.*

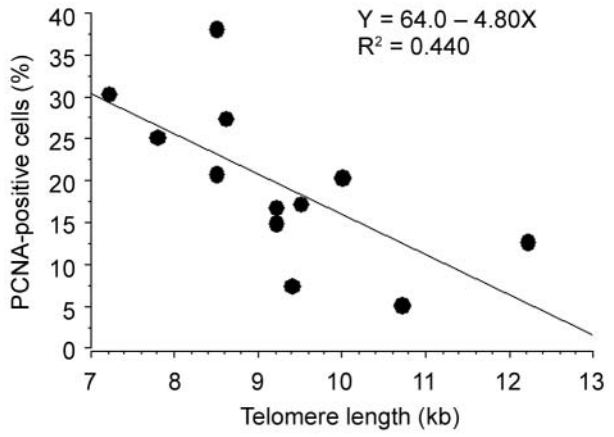

Figure 2. *Correlation between telomere length of tumor and PCNA-positive cell staining.*

Telomerase activity and clinical results. Telomerase activity was detectable in 75% (9 of 12 samples) of the tumor samples (Table I). The levels of detectable telomerase activity were between 0.05 and 0.51 TPG. There was a significant correlation between telomerase activity and age at surgery (r=0.678, p=0.015) (Figure 3). There was no significant correlation between telomerase activity and telomere length (r=0.263, p=0.410), tumor size (r=0.324, p=0.305), and on gender (p=0.094).

Recurrent cases analysis with telomere length, telomerase activity and PCNA-stained tumor cells. Regarding recurrent cases, telomere lengths increased, and the levels of telomerase activity decreased with recurrence (Figure 4, Table I). PCNA-positive cells were significantly higher in

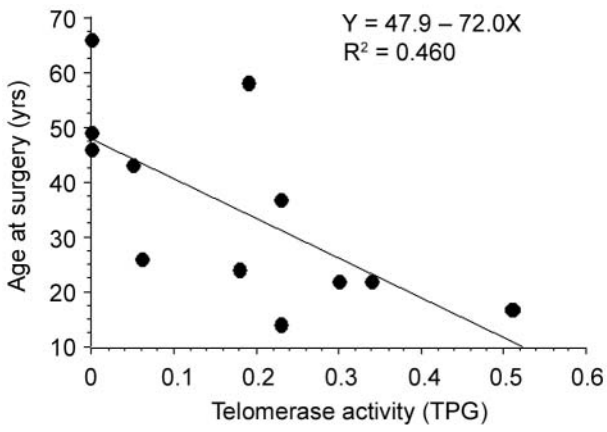

Figure 3. *Correlation between telomerase activity and age at surgery.*

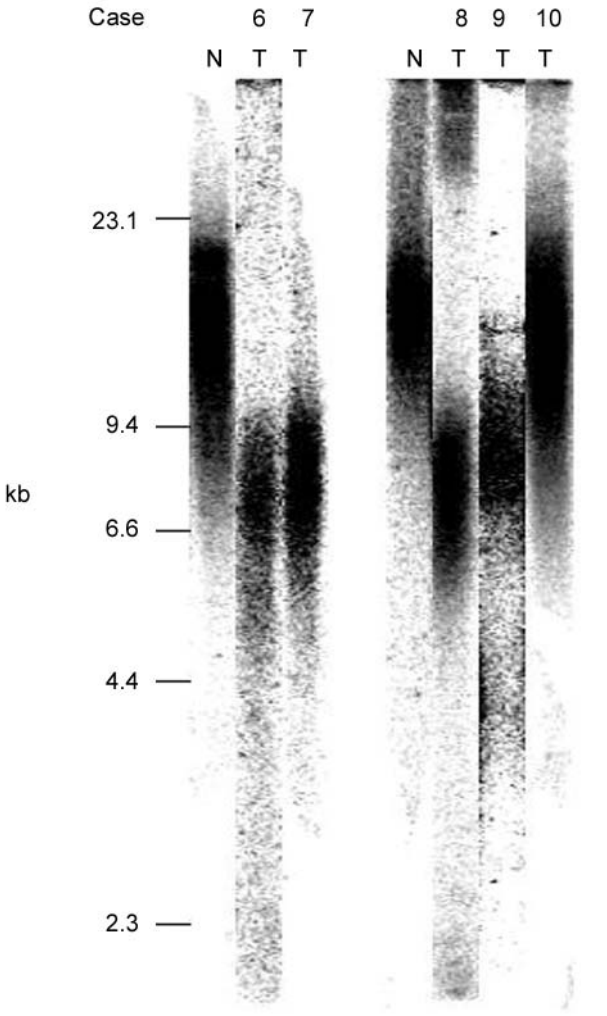

Figure 4. *Southern hybridization analysis of the telomere region in desmoid tumor paired with normal tissue DNA from the same patient (recurrent cases). Telomere lengths increased with recurrence.N: normal tissue; T: tumor sample.*

case 6 (patient F, initial tumor, 25.2±4.2%) than case 7 (patient F, recurrent tumor, 16.8±2.6%) (p=0.014) (Table I). Case 8 (patient G, initial tumor, 38.1±12.9%) had a significantly higher proportion of PCNA-positive cells than case 9 (patient G, first recurrent tumor, 7.5±2.9%) (p=0.0003, Fisher LSD test) and case 10 (patient G, second recurrent tumor, 5.2±1.4%) (p=0.0002, Fisher LDS test). There was no significant difference between case 9 and case 10 regarding PCNA-positive cells (p=0.690, Fisher LSD) (Table I). Patient F had a recurrence after the last operation. However, tumor size had not increased on MR Imaging over 4 years and 6 months. Patient G has had no local recurrence since the final operation.

Discussion

A desmoid tumor, which is a histologically benign soft tissue tumor, has a locally aggressive character and its local recurrence is high despite complete tumor resection (2, 11, 12). In contrast, both spontaneous regression and natural disappearance after surgery have been reported in some cases (1, 2).

The several etiologies of this tumor have been reported as follows: an abnormal expression of c-sis and PDGF was identified, and proposed a mechanism by which inappropriate expression of c-sis increases production of PDGF, which can be a mitogen for fibrocytes (13); elevated beta-catenin protein, caused by a somatic APC mutation resulting in a truncated protein in some cases, was present (14); the tumor suppressor gene Rb1 has been shown to have decreased expression (15). However, the prediction of the clinical behaviour of this tumor is impossible (16).

Many telomere-related studies have been reported in human carcinomas for diagnostic and/or prognostic utilities. Telomerase activity has been reported in 80-90% of carcinomas and in some types of carcinomas high telomerase activity has been reported as a marker of tumor aggressiveness (8, 9, 17, 18). In addition, there has been a considerable shortening of telomeres despite the expression of telomerase in many cancer cells (3, 4, 8, 9). In contrast, although some benign tumors also have detectable telomerase activity, almost no benign tumors express telomerase activity and alteration of telomere length (5, 19). Middleton *et al.* reported no significant difference between desmoid samples and their controls (20). In our study, there was also no significant difference between telomere lengths of tumors and those of normal tissues (p=0.065). Scates *et al.* reported that no telomerase activity was detected in desmoids (21). In contrast, nine (75%) out of 12 desmoid samples analyzed exhibited low levels of telomerase activity. That some desmoids had telomerase activity cannot be easily explained because the pathogenesis of desmoid tumor is not well understood. In this study, there was a significant correlation between telomere length of tumor and tumor size as measured with MR images (p=0.049), with telomeres being shorter with increased tumor size. In addition, there was a significant correlation between telomere length and PCNA-positive cell staining with telomere being shorter with increased positive PCNA-staining (p=0.017). This shortening in telomere length may be a result of increased length of time of tumor cell division for tumor expansion and increased tumor cell aggressiveness. Interestingly, it has been reported that recurrent cases had stabilized and naturally decreased in size, and when left alone, the recurrent tumor had not created difficulties with the patient's level of activity (1, 2). Regarding our recurrent cases, telomere lengths increased. In addition, telomerase activities and PCNA-positive staining cells indicating cell proliferation decreased with recurrence. In some reports, when telomerase activity was insufficient, tumor cell telomeres eventually reached a critical length and stopped proliferation (8). However, in our cases, telomere lengths slightly increased with each recurrence. The presence of low telomerase activity with a low cell division rate might increase the length of telomeres. It is expected that in tumors without telomerase expression, tumor aggressiveness is lost. For example, recurrent cases 7 and 10 may have no aggressive potential for tumor proliferation and, therefore, would not require additional treatment. There are some reports in which the average age of patients with a recurrent tumor was lower than that of patients with desmoid tumor, and the duration of tumor activity was longer in younger patients (2, 11). In the present study, there was a significant correlation between telomerase activity and age (p=0.015), with younger patients expressing higher telomerase activity. These results may explain the higher recurrence rates in younger patients.

In summary, we have shown that decreasing telomere length correlates with desmoid tumor size, probably due to the fact that at increased length of time the tumor undergoes proliferations. With recurrence, telomere length increased and telomerase activity and PCNA-staining decreased. These results may be due to a low cell division rate, and the presence of telomerase activity, despite very low levels. Telomere length and presence of telomerase activity may be markers of oncological features of desmoid tumors. Recurrent lesions that have no telomerase expression need only to be followed by sequential assessments because these cases are expected to stabilize, naturally decrease in size and not create difficulties with the patient's quality-of-life.

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