Telomere shortening and expression of TRF1 and TRF2 in uterine leiomyoma

BONG-KYEONG OH[1], YOOJUNG CHOI[2] and JOONG SUB CHOI[1,2]

[1]Institute for the Integration of Medicine and Innovative Technology; [2]Department of Obstetrics and Gynecology, Hanyang University College of Medicine, Seoul 04763, Republic of Korea

Received July 24, 2020; Accepted November 5, 2020

DOI: 10.3892/mmr.2021.12243

Abstract. Uterine leiomyoma is a benign smooth muscle tumor of the uterus that can exhibit histopathological traits that mimic malignancy. Telomere shortening is an early event in tumorigenesis and telomerase activation facilitates tumor progression later in the course of carcinogenesis. Telomeric repeat-binding factor (*TRF*)*1* and *TRF2* protect telomeres, and their gene expression levels are dysregulated in various cancer types. However, the roles of telomeres and telomere protection proteins in uterine leiomyoma remain largely unknown. In this study, telomere length and the mRNA levels of various telomere-related genes in normal tissues and leiomyoma were determined, and their relationships were evaluated. Uterine leiomyoma and normal myometrium were surgically obtained from 18 and 13 patients, respectively. Telomere length and gene expression were determined by Southern blot analysis and reverse transcription-quantitative PCR, respectively. In matched samples, telomeres were consistently shorter in leiomyoma tissue than in adjacent normal tissue. *TRF1*, *TRF2*, PIN2-interacting telomerase inhibitor 1 (*PINX1*), and telomerase RNA component were expressed at comparable levels in both leiomyoma and normal tissues. None of these genes were associated with telomere length in leiomyoma. All tested tissues were negative for telomerase reverse transcriptase, which encodes the catalytic component of telomerase, indicating that cells in uterine leiomyoma were not immortalized. In summary, telomere erosion, which reflects active proliferation during tumor evolution, was evident in uterine leiomyoma. Steady-state expression of *TRF1*, *TRF2* and *PINX1* may be important for maintenance of telomere integrity in leiomyoma, where telomere length is shortened.

Correspondence to: Dr Bong-Kyeong Oh, Institute for the Integration of Medicine and Innovative Technology, Hanyang University College of Medicine, 222 Wangsimni-ro, Seongdong-gu, Seoul 04763, Republic of Korea
E-mail: bongoh0401@hanyang.ac.kr

Dr Joong Sub Choi, Department of Obstetrics and Gynecology, Hanyang University College of Medicine, 222 Wangsimni-ro, Seongdong-gu, Seoul 04763, Republic of Korea
E-mail: choiyjjy1@hanyang.ac.kr

Key words: uterine leiomyoma, telomere, telomere proteins

Introduction

Uterine leiomyoma, also known as uterine fibroid, is the most common benign tumor, affecting ~70% of women by the age of 50 (1). These lesions disrupt the function of the uterus and cause several health complications, such as irregular bleeding, recurrent pregnancy loss, and pelvic discomfort (2). Although pathogenetic factors such as genetics, epigenetics, microRNA, ovarian steroids, and growth factors have been implicated in the development of leiomyoma (1), the underlying pathogenesis remains poorly understood.

Telomeres protect chromosome ends from unnecessary DNA repair and nucleolytic degradation (3). Human somatic cells lose telomeres progressively over the course of cell divisions, and cells with critically short telomeres stop dividing and enter senescence (4). Telomere shortening is thought to be a tumor suppressor mechanism (5), and immortal cells, such as stem cells and transformed cells, express telomerase, thereby maintaining their telomere length (6).

Telomerase is a ribonucleoprotein consisting of two essential components, telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC), and several accessary proteins. TERT, the catalytic protein component, is expressed in stem cells and most cancer cells (7), whereas TERC functions as a template for the synthesis of telomeric repeats, and is ubiquitously expressed in both primary and immortal cells (8). The telomere contains tandem TTAGGG repeats that are associated with a multiprotein complex known as shelterin (3). Telomeric repeat-binding factor (TRF)1 and TRF2 are components of shelterin, which directly bind TTAGGG repeats, protect telomeres, and contribute to telomere maintenance by acting as negative regulators of telomere length (9,10).

Altered expression of *TRF1* and *TRF2* is frequently observed in cancer. In humans, *TRF1* and *TRF2* are induced in hepatitis-induced carcinoma (11), renal cell carcinoma (12), and lung cancer (13). Recently, upregulation of *TRF1* and *TRF2* was also detected in patients with obesity (14). By contrast, these genes are downregulated in breast cancer (15). In addition, *TRF1* is highly expressed in adult stem cells and pluripotent stem cells (16), where it plays a crucial role in maintenance of tissue homeostasis and pluripotency by protecting

telomeres regardless of telomere length (17). PIN2-interacting telomerase inhibitor 1 (PINX1), a TRF1-interacting protein, is a potent telomerase inhibitor and tumor suppressor that is essential for maintaining telomerase activity and chromosome stability (18). Expression of *PINX1* is reduced in several types of cancers, including breast (18) and colorectal tumors (19), and correlates with poor prognosis in patients with ovarian cancer (20). Meanwhile, *PINX1* is upregulated in esophageal squamous cell carcinoma, cervical squamous cell carcinoma (21), and glioma (22). Abnormal regulation of *PINX1* is complex, and the function of this protein in tumorigenesis remains unresolved.

Although uterine leiomyoma are benign tumors, some exhibit histopathological traits that mimic malignancy, including hypercellularity, active mitotic activity, and abnormal nuclei (23). Telomere shortening has been reported in uterine leiomyoma (24,25). However, the expression levels of telomeric repeat-binding proteins and telomerase, as well as their relationship with telomere length, have not been evaluated in uterine leiomyoma. To address this, telomere length and mRNA levels of genes involved in telomere function were analyzed in normal myometrium and uterine leiomyoma, and their relationships were examined.

Materials and methods

Tissue samples. Uterine leiomyoma tissue samples were obtained from 18 female patients (mean age, 45.7±3.03 years; age range, 37-50 years) who underwent myomectomy or hysterectomy for uterine leiomyoma at Hanyang Hospital in Seoul (South Korea) between October 2017 and June 2019; all patients had multiple leiomyoma. Normal myometrium tissues were obtained from 13 of 18 patients. Inclusion criteria included the presence of a symptomatic myoma. Patients with organ dysfunction, for example, in the liver, kidney and lungs, were excluded. Samples taken from the centers of leiomyoma and from adjacent normal myometrium were immediately snap-frozen and stored in liquid nitrogen. This study was approved by the Institutional Review Board of Hanyang Hospital, Hanyang University College of Medicine (approval no. 201707012), and the requirement for informed consent was waived.

Isolation of genomic DNA. Fresh tissue homogenized using a disposable homogenizer (BioMasher; Nippi, Inc.) was incubated with 20 μg DNase- and protease-free RNase A (Thermo Fisher Scientific, Inc.) at 37˚C for 1 h in 500 μl lysis buffer (10 mM Tris-HCl; pH 8.0; 100 mM EDTA; 0.5% (w/v) SDS), then further digested at 50˚C overnight with 50 μg proteinase K (Invitrogen; Thermo Fisher Scientific, Inc.). Genomic DNA was extracted using phenol/chloroform/isoamyl alcohol (PCI; Bioneer Corporation) a total of three times. MaXtract High-Density tubes (Qiagen GmbH) were used for PCI extractions to prevent carryover of organic solvent, proteins, and other contaminants. The supernatant was supplemented with ammonium acetate to a final concentration of 2.5 M and one volume of isopropanol. Genomic DNA spooled on a pipette tip was washed with 70% ethanol three times, air-dried and resuspended in water. Quantification of DNA was performed using a NanoDrop™ 2000 spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.).

Preparation of digoxigenin (DIG)-labeled probes. Oligonucleotides were labeled at the 3'-end by incorporation of a single DIG-labeled ddUTP (Roche Diagnostics). Briefly, 100-200 pmol oligonucleotides were incubated at 37˚C for 60 min with 1 μl of 1 mM DIG-ddUTP and 20 units terminal deoxynucleotidyl transferase (Roche Diagnostics) in 1X reaction buffer and 5 mM $CoCl_2$ in a total volume of 20 μl, and the reaction was stopped by addition of 2 μl of 0.2 M EDTA (pH 8.0).

Southern blot analysis of terminal restriction fragment lengths. Southern hybridization was performed to measure telomere length. Briefly, 5 μg *Hin*FI-cut DNA was fractionated on an 0.8% (w/v) agarose gel and blotted by capillary transfer onto a nylon membrane (Hybond™ N+; GE Healthcare Biosciences) in 10X saline-sodium citrate (SSC) buffer. Blots were UV-crosslinked (Spectronics), pre-hybridized in DIG Easy Hyb (Roche Diagnostics) at 45˚C for 1 h, and hybridized with DIG-labeled $d(TTAGGG)_4$ at the same temperature overnight. The membrane washing and antibody reaction were carried out at room temperature as follows. The membranes were washed in 2X SSC with 0.1% SDS for 15 min twice, in 0.5X SSC with 0.1% SDS for 15 min twice, and then in 1X maleic acid buffer (MAB; 0.1 M maleic acid; 0.15 M NaCl; pH 7.5) for 5 min. The blots were immersed in 1X Blocking Solution (Roche Diagnostics; diluted in 1X MAB) for 30 min, then incubated with an anti-DIG antibody conjugated to alkaline phosphatase (cat. no. 11093274910; 1:10,000; Roche; MilliporeSigma; Merck KGaA) in 1X Blocking Solution for 60 min. The membranes were washed in 1X washing buffer (0.3% Tween-20 in 1X MAB) for 15 min twice, then in detection buffer (0.1 M NaCl; 0.1 M Tris-HCl, pH 9.5) at room temperature for 5 min. CDP-Star (Roche Diagnostics) was used as a chemiluminescence substrate, and the hybridization signal was detected by scanning using a ChemiDoc XRS+ image analysis system (Bio-Rad Laboratories, Inc.). For the next round of hybridization, the membrane was rinsed thoroughly with sterile water, washed at 37˚C in stripping buffer (0.2 N NaOH; 0.1% SDS) for 15 min twice, then rinsed with 2X SSC buffer for 5 min. The de-probed blot was rehybridized with the DIG-labeled $d(CAC)_8$ probe. Telomere length (TL) was calculated as previously described (26). Briefly, the telomere signal in each lane was quantified in a grid object defined as a single column with 20 rows (1x20 grid over the lane ranging from 3.5 to 21 kb), using the Image Lab software (version 6; Bio-Rad Laboratories, Inc.), and the TL was defined as $\sum(MW_i \times OD_i)/\sum(OD_i)$, where OD_i is the optical density and MW_i is the molecular weight of the DNA at the i^{th} position.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from tissues using Tri-RNA (Favorgen) or TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Then, 1 μg RNA was incubated with 2 units RNase-free DNase I (New England Biolabs, Inc.) and 40 units RNase inhibitor (Takara Bio, Inc.) with 2 mM DTT in a total volume of 1 μl at 37˚C for 30 min, heat-inactivated at 75˚C for 10 min, then subjected to cDNA synthesis. First-strand cDNA synthesis was carried out in 20-μl volumes containing 6 μM random hexamers (Takara Bio, Inc.) and 200 units

Table I. Primers used for reverse transcription-quantitative PCR.

First author, year	Primer	Sequence (5'-3')	(Refs.)
Scheibe *et al*, 2013	*TRF1*-forward	TGCTTTCAGTGGCTCTTCTG	(27)
Scheibe *et al*, 2013	*TRF1*-reverse	ATGGAACCCAGCAACAAGAC	(27)
Scheibe *et al*, 2013	*TRF2*-forward	TTGTGGGGTCCTTGGACATA	(27)
Scheibe *et al*, 2013	*TRF2*-reverse	CCAGTAGAAAACTGGTCAAGGAA	(27)
Present study	*PINX1*-forward	CACTCCAGAGGAGAACGAAACC	-
Present study	*PINX1*-reverse	CACCGGCTTGGCAAAGTACT	-
Park *et al*, 2008	*TERT*-forward	TGTGCACCAACATCTACAAG	(28)
Park *et al*, 2008	*TERT*-reverse	GCGTTCTTGGCTTTCAGGAT	(28)
Lundberg *et al*, 2002	*TERC*-forward	TCTAACCCTAACTGAGAAGGGCGTAG	(29)
Lundberg *et al*, 2002	*TERC*-reverse	GTTTGCTCTAGAATGAACGGTGGAAG	(29)
Deng *et al*, 2012	*GAPDH*-forward	AGCCACATCGCTCAGACAC	(30)
Deng *et al*, 2012	*GAPDH*-reverse	GCCCAATACGACCAAATCC	(30)

TRF, telomeric repeat-binding factor, PINX1, PIN2 (TERF1) interacting telomerase inhibitor 1; TERT, telomerase reverse transcriptase; TERC, telomerase RNA component.

Moloney murine leukemia virus (M-MLV) reverse transcriptase (Thermo Fisher Scientific, Inc.) at 37˚C for 50 min. To ensure that genomic DNA was completely removed, the RT reaction mixture without M-MLV reverse transcriptase was subjected to PCR with *TERC*-specific primers. PCR was performed in duplicate with 1 μl cDNA template, 0.2 μM primers (Macrogen), and 1X LightCycler® 480 SYBR-Green I Master mix (Roche Diagnostics) using the LightCycler® 480 system (Roche Diagnostics). Thermocycling conditions were as follows: i) 95˚C for 5 min; ii) 40 cycles of 95˚C for 15 sec and 60˚C for 1 min; iii) a dissociation stage of 95˚C for 5 sec; 65˚C for 1 min; and iv) 40˚C for 30 sec. *GAPDH* was used as the reference gene, and sequences of the primers used in the present study are listed in Table I (27-30). Expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (31).

Statistical analysis. The data were obtained from a single experiment and data are presented as the mean ± standard deviation. Statistical analysis was performed using SPSS software (v. 24, IBM, Corp.) and assessed using an unpaired Student's t-test and the Pearson correlation. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Telomere shortening in uterine leiomyoma. In this study, terminal restriction fragment lengths were measured using Southern blotting in leiomyoma (n=18) and adjacent normal myometrium (n=13) tissue. Of note, five of the leiomyoma did not have matched normal tissues (Table II). Hybridization with the telomere-specific 3'-DIG-labeled d(TTAGGG)$_4$ probe indicated that telomere length ranged from 10.4 to 14.9 kb in normal myometrium and from 8.7 to 12.5 kb in leiomyoma (Fig. 1 and Table II). Leiomyoma samples presented shorter telomeres than the normal myometrium (Fig. 1B). Mean telomere lengths in normal and leiomyoma tissue samples were 12.5±1.11 (n=13) and 11.1±1.04 kb (n=18), respectively ($P<0.001$; Fig. 1B and Table II). There were no significant

Table II. Telomere length in normal myometrium and leiomyoma tissue.

Patient no.	Telomere length, kb		Telomere shortening[a], kb	Age, years
	Normal	Leiomyoma		
1	13.6	11.6	-2.0	44
2	13.1	12.2	-0.9	45
3	11.6	8.7	-2.9	47
4	12.4	11.1	-1.3	37
5	12.7	12.5	-0.2	48
6	11.6	9.6	-2.0	47
7	12.0	10.7	-1.3	48
	12.3*	10.1*		
8	12.7	12.2	-0.5	50
	12.1*	11.7*		
		11.4*		
9	11.9	11.4	-0.5	49
	12.5*	11.8*		
		12.2*		
10	10.4	9.1	-1.3	46
11	12.1	11.6	-0.5	48
12	14.9	11.4	-3.5	45
13	13.2	11.7	-1.5	44
14		11.1		43
15		10.5		43
16		11.2		44
17		10.8		48
18		11.8		47
Total	12.5±1.11[b] (n=13)	11.1±1.04[b] (n=18)	-1.4±0.98[b] (n=13)	45.7±3.03[b] (n=18)

[a]Telomere length in leiomyoma-telomere length in normal tissue; [b]mean ± SD, *, excluded in mean value.

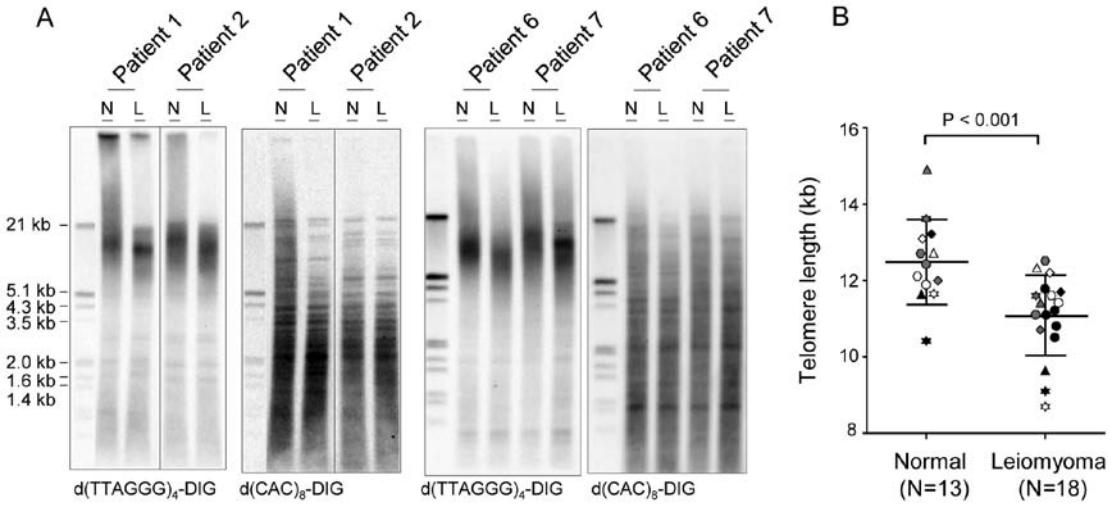

Figure 1. Telomere shortening in uterine leiomyoma. (A) Southern blot analysis of telomeres. Terminal restriction fragment lengths were detected in uterine leiomyoma and adjacent normal myometrium, indicated by L and N, respectively. The blot was hybridized with a DIG-labeled d(TTAGGG)$_4$ probe, stripped, and then reprobed with a DIG-labeled d(CAC)$_8$ probe. Size markers are indicated on the left-hand side. Two representative blots are shown. Note that DNA samples from patients 1 and 2 were run on the same gel, but not side by side. (B) Telomere length in uterine leiomyoma and normal myometrium. Patients are represented by different symbols. Five leiomyoma without matched normal tissues are shown in black circles. Horizontal lines and error bars represent mean telomere length and standard deviation in each group, respectively. Unpaired Student's t-test was used to compare differences in telomere length between normal and leiomyoma tissues. DIG, digoxigenin.

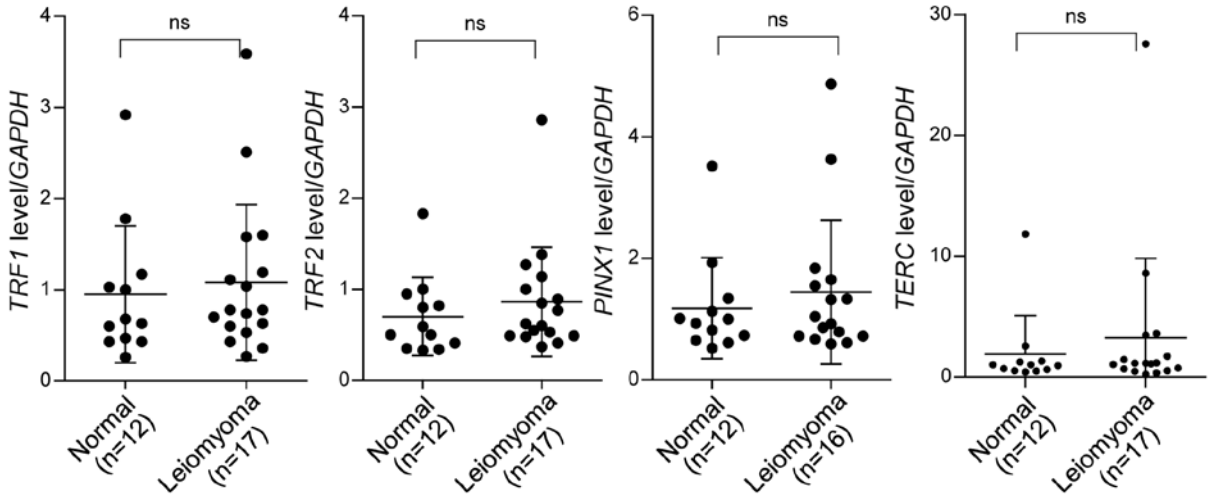

Figure 2. Gene expression in normal myometrium and uterine leiomyoma. DNase I-treated total RNA was reverse transcribed and subjected to quantitative PCR using gene-specific primers. *GAPDH* was used as a reference gene to normalize RT-qPCR data. Horizontal lines and error bars indicate mean values and standard deviation in each group, respectively. Data were analyzed using an unpaired Student's t-test. A pair of tissues (normal and leiomyoma) from a patient (no. 12 in Table II) were excluded in the analysis because of the poor RNA quality, and one additional leiomyoma sample (no. 15 in Table II) was excluded in the qPCR detection for PINX1 due to an experimental error. TRF, telomeric repeat-binding factor; PINX1, PIN2 (TERF1) interacting telomerase inhibitor 1; TERC, telomerase RNA component; ns, not significant.

differences in telomere length related to patient age, possibly due to the narrow range of patient ages (37-50 years) (data not shown). Telomere lengths in multiple leiomyoma samples from a single patient (Table II; patients no. 7, 8, 9) were also measured, but no obvious variations in lengths was observed among measurements. Membranes re-probed with a 3'-DIG-labeled d(CAC)$_8$ probe for minisatellite DNA confirmed the integrity of genomic DNA. A minisatellite is a tract of repetitive DNA sequences that are present throughout the entire genome and often used for the fingerprinting of DNA (32). Indeed, hybridization with d(CAC)$_8$ probe showed different hybridization patterns by patients (Fig. 1A).

Expression of TRF1, TRF2 and PINX1 in leiomyoma. TRF1 and TRF2, components of shelterin, protect telomeres and regulate telomere length. Depletion or deletion of these genes induces a persistent DNA damage response at telomeres, resulting in cessation of cell division and induction of apoptosis or senescence (16,33-35). Meanwhile, overexpression of *TRF1*, *TRF2* and *PINX1* results in telomere shortening in telomerase-positive cells (9,10,36), ultimately leading to induction of cell crisis which is characterized by the reduction in growth rate. To determine whether the expression of *TRF1*, *TRF2* and *PINX1* varied during the development of leiomyoma, the mRNA levels of these genes in leiomyoma (n=17)

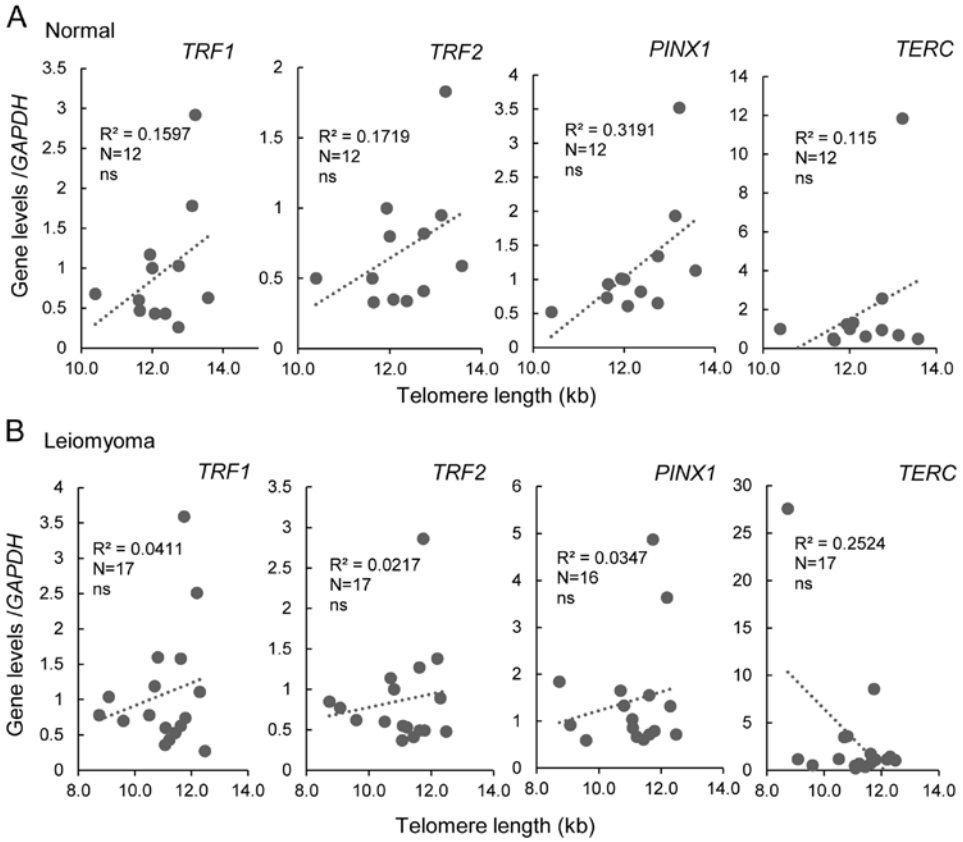

Figure 3. Correlation analysis of telomere-associated gene expression with telomere length in normal myometrium and leiomyoma. Pearson correlation was used to assess correlations between mRNA levels and telomere length in (A) normal myometrium and (B) leiomyoma. ns, not significant. TRF, telomeric repeat-binding factor; PINX1, PIN2 (TERF1) interacting telomerase inhibitor 1; TERC, telomerase RNA component.

and normal tissues (n=12) were determined. RT-qPCR results suggested that these genes were expressed at similar levels in normal and leiomyoma nodules (Fig. 2). To determine whether there was a possible association between gene expression and telomere length, Pearson's correlation was used (Fig. 3). The expression of *TRF1*, *TRF2* and *PINX1* did not correlate with telomere length in either normal myometrium or leiomyoma (Fig. 3). Moreover, the mRNA levels of *TERT* and *TERC*, the essential components of telomerase, were also measured. *TERC* was expressed at comparable levels in leiomyoma and normal tissue samples, independently of telomere length (Figs. 2 and 3), whereas *TERT* expression was negative in all samples tested (data not shown).

Discussion

Uterine leiomyoma accounts for the majority of hysterectomies and is associated with substantial morbidity, such as excessive uterine bleeding, anemia, pelvic discomfort and recurrent pregnancy loss, in women of reproductive age (1). Development of leiomyoma proceeds through a multistep process involving the transformation of normal myocytes into abnormal ones and their growth into tumors (1). Accumulating evidence suggests that some intrinsic abnormalities of the myometrium, abnormal myometrial receptors for estrogen, and hormonal changes or altered responses to ischemic damage during the menstrual period may be responsible for the initiation of (epi)genetic changes found in uterine myoma (37,38). However, the pathogenesis of leiomyoma remains largely unclear. In this study, telomere shortening was clearly identified as a key event associated with leiomyoma. Leiomyoma samples displayed shorter telomeres, ranging from a few hundred bases to several kilobases, than adjacent normal tissues. However, most leiomyoma had telomeres of ≥10 kb. In fact, critically short telomeres <5 kb, which are frequently found in malignant tumors (11), were not detected in leiomyoma. These observations suggested that significant levels of cell division occur during progression to leiomyoma, but telomeres are long enough to maintain their integrity within the tumors.

Southern blot analysis was employed in this study to measure telomere length, and the use of this technique is limited in studies involving large numbers of samples. The quantitative PCR technique which provides relative telomere length (RTL) and requires a small number of cells is widely used in epidemiological studies (39,40). In fact, the RTLs have been successfully measured in circulating serum DNA from patients with endometrial cancer (41,42). Further study is needed to assess the RTL in the serum of a large number of uterine leiomyoma patients, which will provide a better understanding of whether telomere length is a diagnostic marker for early detection of uterine leiomyoma.

There were no significant alterations in the levels of *TRF1*, *TRF2* or *PINX1* in uterine leiomyoma, and the expression of these genes did not correlate with telomere length. Our findings are in accordance with a previous study that reported

low but constant levels of *TRF1* and *TRF2* expression at early stages in human hepatocarcinogenesis (normal, chronic hepatitis, liver cirrhosis, and large regenerative nodule) and shortening of telomeres with disease progression (11). It may be hypothesized that the steady-state expression of telomere protection genes such as *TRF1*, *TRF2* and *PINX1* is important for the maintenance of telomere integrity in benign tumors, which may impede tumor progression to malignancy. In addition, unsurprisingly, leiomyoma remained telomerase-negative as revealed by RT-qPCR for *TERT*, confirming that cells in this tumor did not acquire immortality. Several studies have demonstrated that *TERC* levels are upregulated in early preneoplastic stages (43-45). This phenomenon, however, was not detected in leiomyoma progression. *TERC* is essential for telomere maintenance in telomerase-positive cells, but the telomerase- and telomere-independent functions of *TERC* remain elusive. *TERC* may function as a noncoding RNA that prevents apoptosis in normal telomerase-negative cells (46).

In conclusion, expression levels of genes essential for telomere protection were maintained during neoplastic transformation of myometrium to leiomyoma, and telomere shortening was evident during this process. Persistent expression of telomere protection genes may lead to maintenance of telomere integrity in leiomyoma. These results provide insight into the progression of normal tissue to benign tumors.

Acknowledgements

We thank Dr Baikseol Cho (Department of Obstetrics and Gynecology, Hanyang University College of Medicine, Seoul, South Korea) for her valuable input on Institutional Review Board approval.

Funding

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (grant no. 2019R1I1A1A01041367) and the Ministry of Science and Information and Communication Technology (grant no. 2017R1A2B4004721).

Availability of data and materials

The data are available from the corresponding author on reasonable request.

Authors' contributions

BKO and JSC conceived and designed the study and BKO drafted the manuscript. BKO and YC performed the experiments and analyzed the data. All authors confirm the authenticity of all the raw data, and read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Hanyang Hospital, Hanyang University College of Medicine (approval no. 201707012). The requirement for informed consent was waived.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bulun SE: Uterine fibroids. N Engl J Med 369: 1344-1355, 2013.
2. Al-Hendy A, Myers ER and Stewart E: Uterine Fibroids: Burden and Unmet Medical Need. Semin Reprod Med 35: 473-480, 2017.
3. de Lange T: Shelterin-Mediated Telomere Protection. Annu Rev Genet 52: 223-247, 2018.
4. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL and Shay JW: Specific association of human telomerase activity with immortal cells and cancer. Science 266: 2011-2015, 1994.
5. Shay JW and Wright WE: Role of telomeres and telomerase in cancer. Semin Cancer Biol 21: 349-353, 2011.
6. Counter CM, Hirte HW, Bacchetti S and Harley CB: Telomerase activity in human ovarian carcinoma. Proc Natl Acad Sci USA 91: 2900-2904, 1994.
7. Nakayama J, Tahara H, Tahara E, Saito M, Ito K, Nakamura H, Nakanishi T, Tahara E, Ide T and Ishikawa F: Telomerase activation by hTRT in human normal fibroblasts and hepatocellular carcinomas. Nat Genet 18: 65-68, 1998.
8. Avilion AA, Piatyszek MA, Gupta J, Shay JW, Bacchetti S and Greider CW: Human telomerase RNA and telomerase activity in immortal cell lines and tumor tissues. Cancer Res 56: 645-650, 1996.
9. van Steensel B and de Lange T: Control of telomere length by the human telomeric protein TRF1. Nature 385: 740-743, 1997.
10. van Steensel B, Smogorzewska A and de Lange T: TRF2 protects human telomeres from end-to-end fusions. Cell 92: 401-413, 1998.
11. Oh BK, Kim YJ, Park C and Park YN: Up-regulation of telomere-binding proteins, TRF1, TRF2, and TIN2 is related to telomere shortening during human multistep hepatocarcinogenesis. Am J Pathol 166: 73-80, 2005.
12. Pal D, Sharma U, Singh SK, Kakkar N and Prasad R: Over-expression of telomere binding factors (TRF1 & TRF2) in renal cell carcinoma and their inhibition by using SiRNA induce apoptosis, reduce cell proliferation and migration invitro. PLoS One 10: e0115651, 2015.
13. Nakanishi K, Kawai T, Kumaki F, Hiroi S, Mukai M, Ikeda E, Koering CE and Gilson E: Expression of mRNAs for telomeric repeat binding factor (TRF)-1 and TRF2 in atypical adenomatous hyperplasia and adenocarcinoma of the lung. Clin Cancer Res 9: 1105-1111, 2003.
14. Grun LK, Teixeira ND Jr, Mengden LV, de Bastiani MA, Parisi MM, Bortolin R, Lavandoski P, Pierdoná V, Alves LB, Moreira JC, *et al*: TRF1 as a major contributor for telomeres' shortening in the context of obesity. Free Radic Biol Med 129: 286-295, 2018.
15. Saito K, Yagihashi A, Nasu S, Izawa Y, Nakamura M, Kobayashi D, Tsuji N and Watanabe N: Gene expression for suppressors of telomerase activity (telomeric-repeat binding factors) in breast cancer. Jpn J Cancer Res 93: 253-258, 2002.
16. Schneider RP, Garrobo I, Foronda M, Palacios JA, Marión RM, Flores I, Ortega S and Blasco MA: TRF1 is a stem cell marker and is essential for the generation of induced pluripotent stem cells. Nat Commun 4: 1946, 2013.
17. Bejarano L, Schuhmacher AJ, Méndez M, Megías D, Blanco-Aparicio C, Martínez S, Pastor J, Squatrito M and Blasco MA: Inhibition of TRF1 Telomere Protein Impairs Tumor Initiation and Progression in Glioblastoma Mouse Models and Patient-Derived Xenografts. Cancer Cell 32: 590-607.e4, 2017.
18. Zhou XZ, Huang P, Shi R, Lee TH, Lu G, Zhang Z, Bronson R and Lu KP: The telomerase inhibitor PinX1 is a major haploinsufficient tumor suppressor essential for chromosome stability in mice. J Clin Invest 121: 1266-1282, 2011.
19. Deng W, Jiao N, Li N, Wan X, Luo S and Zhang Y: Decreased expression of PinX1 protein predicts poor prognosis of colorectal cancer patients receiving 5-FU adjuvant chemotherapy. Biomed Pharmacother 73: 1-5, 2015.

20. Cai MY, Zhang B, He WP, Yang GF, Rao HL, Rao ZY, Wu QL, Guan XY, Kung HF, Zeng YX, *et al*: Decreased expression of PinX1 protein is correlated with tumor development and is a new independent poor prognostic factor in ovarian carcinoma. Cancer Sci 101: 1543-1549, 2010.
21. Qian D, Zhang B, He LR, Cai MY, Mai SJ, Liao YJ, Liu YH, Lin MC, Bian XW, Zeng YX, *et al*: The telomere/telomerase binding factor PinX1 is a new target to improve the radiotherapy effect of oesophageal squamous cell carcinomas. J Pathol 229: 765-774, 2013.
22. Bai J, Chen YS, Mei PJ, Liu QH, Du Y and Zheng JN: PinX1 is up-regulated and associated with poor patients' survival in gliomas. Int J Clin Exp Pathol 8: 6952-6959, 2015.
23. Oliva E, Carcangiu M, Carinelli SG, Ip P, Loening T and Longacre TA: Mesenchymal tumours. In: WHO Classification of Tumours of Female Reproductive Organs. 4th edition. Kurman RJ, Carcangiu ML, Herrington CS and Young RH (eds). IARC Press, Lyon, pp135-138, 2014.
24. Bonatz G, Frahm SO, Andreas S, Heidorn K, Jonat W and Parwaresch R: Telomere shortening in uterine leiomyomas. Am J Obstet Gynecol 179: 591-596, 1998.
25. Rogalla P, Rohen C, Hennig Y, Deichert U, Bonk U and Bullerdiek J: Telomere repeat fragment sizes do not limit the growth potential of uterine leiomyomas. Biochem Biophys Res Commun 211: 175-182, 1995.
26. Kruk PA, Rampino NJ and Bohr VA: DNA damage and repair in telomeres: Relation to aging. Proc Natl Acad Sci USA 92: 258-262, 1995.
27. Scheibe M, Arnoult N, Kappei D, Buchholz F, Decottignies A, Butter F and Mann M: Quantitative interaction screen of telomeric repeat-containing RNA reveals novel TERRA regulators. Genome Res 23: 2149-2157, 2013.
28. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW and Daley GQ: Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451: 141-146, 2008.
29. Lundberg AS, Randell SH, Stewart SA, Elenbaas B, Hartwell KA, Brooks MW, Fleming MD, Olsen JC, Miller SW, Weinberg RA, *et al*: Immortalization and transformation of primary human airway epithelial cells by gene transfer. Oncogene 21: 4577-4586, 2002.
30. Deng Z, Wang Z, Stong N, Plasschaert R, Moczan A, Chen HS, Hu S, Wikramasinghe P, Davuluri RV, Bartolomei MS, *et al*: A role for CTCF and cohesin in subtelomere chromatin organization, TERRA transcription, and telomere end protection. EMBO J 31: 4165-4178, 2012.
31. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-ΔΔC(T)) method. Methods 25: 402-408, 2001.
32. Jeffreys AJ, Wilson V and Thein SL: Hypervariable 'minisatellite' regions in human DNA. Nature 314: 67-73, 1985.
33. Karlseder J, Broccoli D, Dai Y, Hardy S and de Lange T: p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science 283: 1321-1325, 1999.
34. Celli GB and de Lange T: DNA processing is not required for ATM-mediated telomere damage response after TRF2 deletion. Nat Cell Biol 7: 712-718, 2005.
35. Martínez P, Thanasoula M, Muñoz P, Liao C, Tejera A, McNees C, Flores JM, Fernández-Capetillo O, Tarsounas M and Blasco MA: Increased telomere fragility and fusions resulting from TRF1 deficiency lead to degenerative pathologies and increased cancer in mice. Genes Dev 23: 2060-2075, 2009.
36. Zhou XZ and Lu KP: The Pin2/TRF1-interacting protein PinX1 is a potent telomerase inhibitor. Cell 107: 347-359, 2001.
37. Laganà AS, Vergara D, Favilli A, La Rosa VL, Tinelli A, Gerli S, Noventa M, Vitagliano A, Triolo O, Rapisarda AM, *et al*: Epigenetic and genetic landscape of uterine leiomyomas: A current view over a common gynecological disease. Arch Gynecol Obstet 296: 855-867, 2017.
38. Yang Q, Mas A, Diamond MP and Al-Hendy A: The mechanism and function of epigenetics in uterine leiomyoma development. Reprod Sci 23: 163-175, 2016.
39. Cawthon RM: Telomere measurement by quantitative PCR. Nucleic Acids Res 30: e47, 2002.
40. Tarik M, Ramakrishnan L, Sachdev HS, Tandon N, Roy A, Bhargava SK and Pandey RM: Validation of quantitative polymerase chain reaction with Southern blot method for telomere length analysis. Future Sci OA 4: FSO282, 2018.
41. Sun Y, Zhang L, Zhao L, Wu X and Gu J: Association of leukocyte telomere length in peripheral blood leukocytes with endometrial cancer risk in Caucasian Americans. Carcinogenesis 36: 1327-1332, 2015.
42. Benati M, Montagnana M, Danese E, Mazzon M, Paviati E, Garzon S, Laganà AS, Casarin J, Giudici S, Raffaelli R, *et al*: Aberrant telomere length in circulating cell-free DNA as possible blood biomarker with high diagnostic performance in endometrial cancer. Pathol Oncol Res 26: 2281-2289, 2020.
43. Blasco MA, Rizen M, Greider CW and Hanahan D: Differential regulation of telomerase activity and telomerase RNA during multi-stage tumorigenesis. Nat Genet 12: 200-204, 1996.
44. Yashima K, Milchgrub S, Gollahon LS, Maitra A, Saboorian MH, Shay JW and Gazdar AF: Telomerase enzyme activity and RNA expression during the multistage pathogenesis of breast carcinoma. Clin Cancer Res 4: 229-234, 1998.
45. Yi X, Tesmer VM, Savre-Train I, Shay JW and Wright WE: Both transcriptional and posttranscriptional mechanisms regulate human telomerase template RNA levels. Mol Cell Biol 19: 3989-3997, 1999.
46. Gazzaniga FS and Blackburn EH: An antiapoptotic role for telomerase RNA in human immune cells independent of telomere integrity or telomerase enzymatic activity. Blood 124: 3675-3684, 2014.