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Increased telomerase activity and human telomerase reverse transcriptase mRNA expression in the endometrium of patients with endometriosis

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BACKGROUND: Endometriosis is considered a frequent, benign disease with the ability to undergo neoplastic processes. The aim of this study was to evaluate the limitless replication potential of the endometrium in patients with endometriosis by examining human telomerase reverse transcriptase (hTERT) mRNA expression and telomerase activity. METHODS: Endometrium samples from 30 endometriosis patients and 30 patients without endometriosis were obtained via endometrial biopsy. The expression of hTERT mRNA was determined by real-time RT–PCR assay, and telomerase activity was measured by telomerase repeat amplification protocol (TRAP) assay. RESULTS: The mean normalized hTERT (N hTERT) mRNA level was significantly higher in the endometriosis than in the control group ($P = 0.013$). The mean hTERT mRNA levels during the proliferative phase and during the secretory phase were higher in the endometriosis group than in the control group, although the difference was only significant for the secretory phase ($P = 0.036$). We found a prominent difference in endometrial telomerase activity between moderate-to-severe endometriosis and the control group ($P = 0.048$). The levels of hTERT mRNA and telomerase activity increased as the disease became more severe ($P = 0.038$, $P = 0.016$). CONCLUSIONS: This study showed the overexpression of hTERT mRNA and telomerase activity in the endometrium of endometriosis patients. These finding suggest that replication potential of endometrial cells may have an important role in the pathogenesis of endometriosis.

Key words: endometriosis/human telomerase reverse transcriptase (hTERT) mRNA/pathogenesis/telomerase

Introduction

Endometriosis is defined as an implantation of endometrial tissues outside their normal location in the uterus. Overall, endometriosis may affect 7–15% of women of reproductive age and usually results in various symptoms such as chronic pelvic pain, dysmenorrhoea and infertility (Lapp, 2000). In infertile women, the prevalence may be as high as 30–40%, and the incidence of this disease is increasing.

In 1927, the pelvic implantation theory by retrograde menstruation of endometrial tissues was first described by Sampson (1927). It is the most widely accepted theory on the pathogenesis of endometriosis. However, no single mechanism can explain all cases of endometriosis, and the pathogenesis is not yet fully understood. Recently, other familial predispositions, immunological factors, cell adhesion factors, angiogenic factors and hormonal factors have been introduced (Wells, 2004). In fact, immunological factors may affect susceptibility to the implantation of endometrial cells, which are shed from the endometrial cavity, and there is much evidence supporting the hypothesis that endometriosis is inherited as a complex genetic trait correlated with susceptibility (Berkkanoglu and Arici, 2003; Kennedy, 2003).

Endometrium from endometriosis patients could have endogenous abnormalities that predispose these patients to the disease, and some genes or gene products are aberrantly expressed in these tissues (Sharpe-Timms, 2001; Giudice and Kao, 2004). Up-regulation of anti-apoptotic genes, such as *bcl*-2, has been noted in the eutopic endometrium of women with

endometriosis (Jones *et al*., 1998). Moreover, Kao *et al*. (2003) have reported that dysregulation of some genes in endometrium of women with endometriosis contributes to implantation failure and infertility.

Although it would be inappropriate to define endometriosis as a premalignant lesion, endometriosis is clinicopathologically similar to neoplastic disease. Endometriosis undergoes malignant transformation such as clear cell or endometrioid ovarian cancer. Also, this disease shares a predisposing factor and molecular similarity with cancer (Varma *et al*., 2004). Angiogenesis, tissue invasion and metastasis are all found in endometriosis patients. Similarly, endometriotic lesions have also displayed an overexpression of anti-apoptotic (*bcl*-2) genes and an underexpression of pro-apoptotic (*bax*) genes (Meresman *et al*., 2000).

Telomeres are repetitive DNA sequences (5′-TTAGGG-3′), at the ends of linear chromosomes, which shorten along with cellular division in somatic cells. Telomerase is a ribonucleoprotein complex and plays an important role in telomere maintenance and cellular immortality (Rhyu, 1995). Normal somatic cells, except hematopoietic stem cells and endometrial cells, do not usually express telomerase. However, telomerase activity has been detected in various cancers and is related to limitless replication potential. Human telomerase reverse transcriptase (hTERT) is a core functional component of telomerase activity. It is a catalytic protein subunit, and a strong correlation has been observed between hTERT mRNA expression and telomerase activity in various tissues (Oshita *et al*., 2000; Baykal *et al*., 2004). It has been proven that hTERT mRNA is a critical determinant of telomerase activity (Kyo *et al*., 1999). Endometrial telomerase activity has been reported to be high during the proliferative phase but is suppressed during the secretory phase of the menstrual cycle. This finding reflects the relationship between telomerase activity and features of the normal endometrium, which is regularly regenerated and has proliferative potential (Yokoyama *et al*., 1998). Lehner *et al*. (2002) reported that telomerase activity and hTERT mRNA levels are significantly higher in endometrial cancer compared with that in the normal, cycling endometrium.

However, no studies concerning telomerase activity in endometriosis patients have been published. Endometriosis is an estrogen-dependent disease with a process similar to that of a neoplastic disease, therefore we hypothesise that increased telomerase activity in the endometrium of endometriosis patients may have a role in the pathogenesis of endometriosis.

In this study, we evaluated the limitless replication potential of the endometrium in women with endometriosis by quantitative examination of hTERT mRNA expression and telomerase activity. Additionally, we analysed the relationship between hTERT mRNA levels and telomerase activities.

Materials and methods

Patients and tissue selection

Fresh surgical specimens from 30 endometriosis patients and 30 normal control tissues were collected via endometrial biopsy. Biopsies were performed during exploration laparotomy or operative laparoscopy at the Department of Obstetrics and Gynecology, Yongdong Severance Hospital, Yonsei University College of Medicine from September 2005 to March 2006. Each sample was immediately frozen and stored at –80°C until use. Ectopic endometrial tissues in the endometriosis group were sent to the Pathology Department, and endometriosis was histologically determined. Informed consent was obtained from all patients, and the study was approved by the institutional review board.

Post-menopausal women, previous hormone or GnRH agonist users and patients who had adenomyosis, endometrial cancer, endometrial hyperplasia or endometrial polyps were excluded.

The endometriosis and control groups were then divided and analysed according to menstrual phases and clinical disease stages established by the revised classification of The American Society for Reproductive Medicine (1997). Endometrial dating was determined from the histology of the endometrium (Noyes *et al*., 1950) and classified into seven phases of the menstrual cycle (menstrual, early-, mid-, late-proliferative and early-, mid-, late-secretory phase). Of 60 women, 8 women were in the early-proliferative phase, 9 were in the mid-proliferative phase, 9 were in the late-proliferative phase, 10 were in the early-secretory phase, 12 were in the mid-secretory and 12 were in the late-secretory phase of their menstrual cycles. Of the 30 women with endometriosis, 13 were in the proliferative phase (3 in the early-, 5 in the mid- and 5 in the late-proliferative phase) and 17 were in the secretory phase (4 in the early-, 7 in the mid- and 6 in the late-secretory phase). Of the 30 control women, 13 were in the proliferative phase (5 in the early-, 4 in the mid- and 4 in the late-proliferative phase) and 17 were in the secretory phase (6 in the early-, 5 in the mid- and 6 in the late-secretory phase). Of the 30 women with endometriosis, 4 were classified as endometriosis stage I, 4 as endometriosis stage II, 11 as endometriosis stage III and 11 as endometriosis stage IV.

Quantification of human hTERT mRNA

Total RNA was isolated from biopsied tissue, using the RNeasy Mini Kit (QIAGEN, Valencia, CA, USA) and following the protocol suggested by the manufacturer. Total RNA (2 μg) was reverse transcribed into cDNA, using the SuperScript™ III first-strand synthesis system (Invitrogen, Carlsbad, CA, USA). Two micrograms of total RNA was mixed with 1 μl Oligo$(dT)_{20}$ primer (0.5 μg/μl), 1 μl dNTPs and deionized water. The total volume of 10 μl was incubated at 65°C for 5 min. After mixing with 2 μl of 10× reverse transcriptase (RT) buffer, 25 mM $MgCl_2$ (4 μl), 0.1 M dithiothreitol (DTT) (2 μl), recombinant RNase inhibitor (RNase OUT TM) (400 U/μl, 1 μl) and SuperScript™ III RT (200 U/μl, 1 μl), each sample was placed in a thermal cycler at 50°C for 50 min; the cycle was stopped at 85°C for 5 min.

The expression of hTERT mRNA was measured by real-time RT–PCR, based on the TaqMan methodology, using a LightCycler® instrument (Roche Diagnostics, Mannheim, Germany). The specific primers and probe nucleotide sequence for hTERT were forward primer 5′-TGACACCTCACCTCACCCAC-3′, reverse primer 5′-CACTGTCTTCCGCAAGTTCAC-3′ and TaqMan probe 5′-(FAM)ACCCTGGTCCGAGGTGTGTCCCTGA(TAMRA)-3. The primers and probe for glyceraldehyde-3-phosphatedehydrogenase (GAPDH) were forward primer 5′-GAAGGTGAAGGTCGGAGTC-3′, reverse primer 5′-GAAGATGGTGATGGGATTTC-3′ and TaqMan probe 5′-(FAM)CAAGCTTCCCGTTCTCAGCC(Tamra)-3′.

For each PCR run, a master mix was prepared on ice with *Taq* polymerase buffer, 4 mmol/l $MgCl_2$, 200 μmol/l deoxynucleotides, 300 nmol/l each primer, 150 nmol/l probe, 1 U *Taq* polymerase and 20 ng cDNA. PCR was performed in a total volume of 20 μl. The thermal cycling conditions included pre-incubation for 10 min at 95°C, followed by 40 cycles of 10 s at 95°C, 30 s at 63°C and 1 s at 72°C.

To normalize the amount of total RNA present in each reaction, we amplified the housekeeping gene *GAPDH*. Our final result was

expressed as a normalized hTERT (N hTERT) mRNA. The amount of target, which was normalized to the endogenous reference (GAPDH) and was compared to the calibrator, was defined by the ΔΔCt method. The normalization formula is target amount = $2^{-\Delta\Delta Ct}$, where ΔΔCt = {[Ct(hTERT sample) – Ct (GAPDH sample)] – [Ct (hTERT calibrator) – Ct (GAPDH calibrator)]}, which was calculated by LightCycler software version 4.0.

Telomerase activity

Telomerase activity was determined according to the telomerase repeat amplification protocol (TRAP) assay that was first described by Kim *et al*. (1994).

Endometrial tissue samples were washed with phosphate-buffered saline (PBS) and then suspended in lysis buffer. Tissues were then homogenized and incubated on ice for 30 min. Cell homogenates were then centrifuged at 14 000 × *g* for 30 min at 4°C. A supernatant 160 µl was recovered, and the protein concentration was measured using a BCA assay kit (Sigma-Aldrich, St. Louis, MO, USA).

The SYBR Green real-time quantitative TRAP assay was conducted with a Quantitative Telomerase Detection kit (EBI, Frederick, MD, USA). The total volume of the reaction mixture was 25 µl, which contained 12.5 µl of quantitative telomerase detection (QTD) premix, 11.5 µl of PCR qualified water and 1.0 µl of tissue extract. About 0.01 µg of protein extract was used for the TRAP assay. The reaction mixture was first incubated at 25°C for 20 min to allow the telomerase in the protein extracts to elongate the TS primer (5′-AATCCGTC-GAGCAGAGTT-3′) by adding a TTAGGG-repeat sequence. After that, PCR was then performed at 95°C for 10 min followed by 45 cycles of amplification at 95°C for 30 s, 60°C for 30 s and 72°C for 30 s on a MiniOpticon real-time PCR machine (Bio-Rad, Hercules, CA, USA). The amplified fluorescence signal in each specimen was measured at the late extension step of each cycle and analysed with Detector software. In the negative control, no fluorescent signal was observed. A standard curve was generated from serial dilutions of telomerase-positive telomerase substrate oligonucleotide (TSR) template (0.5, 0.1, 0.02, 0.004, 0.008, 0.00016, 0.000032 and 0.0000064 µg/ml). The telomerase activity of each specimen was calculated based on the standard curve. One unit of relative telomerase activity (RTA) was defined as the activity equivalent to that in 100 molecules of TSR.

Statistical analysis

Comparison of values between the endometriosis and control groups was done using the Student's *t*-test. The levels of significance in correlation were calculated by Pearson's correlation coefficient. Analysis according to the clinical disease stages in the endometriosis group was done using the Kruskall–Wallis test, because a normal distribution was not obtained. All tests were performed with SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA), and a *P* value <0.05 was considered statistically significant.

Results

Clinical features

A total of 30 samples from each group were examined. The mean age of the endometriosis group was 32.7 ± 6.8 years (mean ± SD) and that of normal control group was 34.6 ± 6.7 years ($P = 0.254$). The body mass index (BMI) and haemoglobin level of each group were not significantly different. The level of serum CA 125 in the endometriosis group (56.5 ± 39.9) was significantly higher than in the control group (20.2 ± 10.5) (Table I).

Table I. Clinical characteristics of the study groups

	Control ($n = 30$)	Endometriosis ($n = 30$)	*P* value
Age (year)	34.6 ± 6.7	32.7 ± 6.8	0.254
BMI (kg/m^2)	22.0 ± 2.3	21.3 ± 2.2	0.240
Hb (g/dl)	12.3 ± 2.3	21.3 ± 2.2	0.785
Serum CA-125 (IU/ml)	20.2 ± 10.5	56.5 ± 39.9	<0.001*

BMI, body mass index, Hb, haemoglobin.
Data are expressed as mean ± SD.
*$P < 0.05$.

Expression of hTERT mRNA

hTERT mRNA expression was detected in 27 of 30 (90%) specimens from the endometriosis group and in 24 of 30 (80%) samples from the normal control group. The mean level of N hTERT mRNA was significantly higher in the endometriosis group than in the control group (52.4 ± 53.7 versus 22.4 ± 24.2, $P = 0.013$) (Table II).

When hTERT mRNA expression of endometrial tissue was analysed according to different phases in all subjects irrespective of disease status, late-proliferative phase showed the maximum expression and the late-secretory phase showed the minimum expression. Nine subjects without expression were all from the late-secretory phase (Figure 1A).

When comparing hTERT mRNA expression according to menstrual phase, the hTERT mRNA levels were highest in the proliferative phase of the endometriosis group and lowest in the secretory phase of the control group (Figure 2A). In both groups, the level of hTERT mRNA was higher in the proliferative phase than in the secretory phase, but no significant differences were found. Although the level of hTERT mRNA during the proliferative was higher in the endometriosis group than in the control group, the difference was also not significant. However, when the hTERT mRNA level during secretory phase in the control group was compared with that in the endometriosis group, it was significantly higher in the latter (17.7 ± 25.1 versus 47.2 ± 40.1, $P = 0.036$).

According to the clinical disease stages in the endometriosis group, hTERT mRNA expression increased by a statistically significant level as the disease became more severe ($P = 0.038$). The mean N hTERT level in the endometriosis group was 10.40 (range 8.85–11.96, median 10.40) in stage I, 16.78 (range 8.75–26.78, median 14.83) in stage II, 47.64 (range 6.07–140.35, median 25.35) in stage III and 74.62 (range 20.17–260.70, median 62.85) in stage IV (Figure 3A).

Table II. Human telomerase reverse transcriptase (hTERT) mRNA expression in the study groups

	Control group	Endometriosis group	*P* value
Positive hTERT mRNA expression (%)	24/30 (80%)	27/30 (90%)	
N hTERT	22.4 ± 24.2	52.4 ± 53.7	0.013*

N hTERT, normalized hTERT.
Data are expressed as mean ± SD.
*$P < 0.05$.

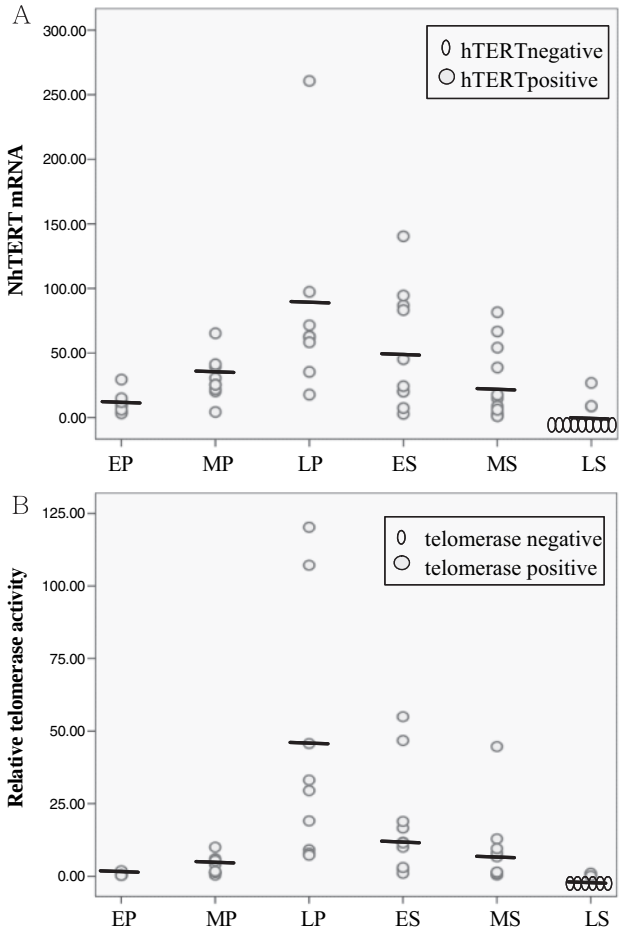

Figure 1. Changes in human telomerase reverse transcriptase (hTERT) mRNA expression and telomerase activity in endometrium of total subjects during the different phases of the menstrual cycle. (**A**) Maximal hTERT mRNA expression was noted in the late-proliferative phase and minimal expression was noted in the late-secretory phase. Nine subjects without expression were all in the late-secretory phase. (**B**) Telomerase activity of endometrium was found to have a similar pattern as hTERT mRNA expression. Six samples without activity were all from the late-secretory phase. EP, early-proliferative phase; ES, early-secretory phase; LP, late-proliferative phase; LS, late-secretory phase; MP, mid-proliferative phase; MS, mid-secretory phase. Black bar: mean value of the phase.

Although slight positive correlation could be seen between hTERT mRNA expression and the endometriotic cyst size in the endometriosis group, there was no significant correlation between the two variables (r = 0.349, P = 0.074). Finally, when comparing the hTERT mRNA expression and the serum CA 125 level, no significant correlation was found (r = 0.193, P = 0.335).

Telomerase activity detection

Telomerase activity was found in 27 of 30 (90%) endometriosis patients and in 27 of 30 (90%) normal patients. The level of RTA was higher in the endometriosis than in the control group, but no significant difference was noted. However, when only the moderate-to-severe endometriosis group was compared with the control group, the difference was statistically significant (21.8 ± 33.0 versus 7.6 ± 13.2, P = 0.048) (Table III).

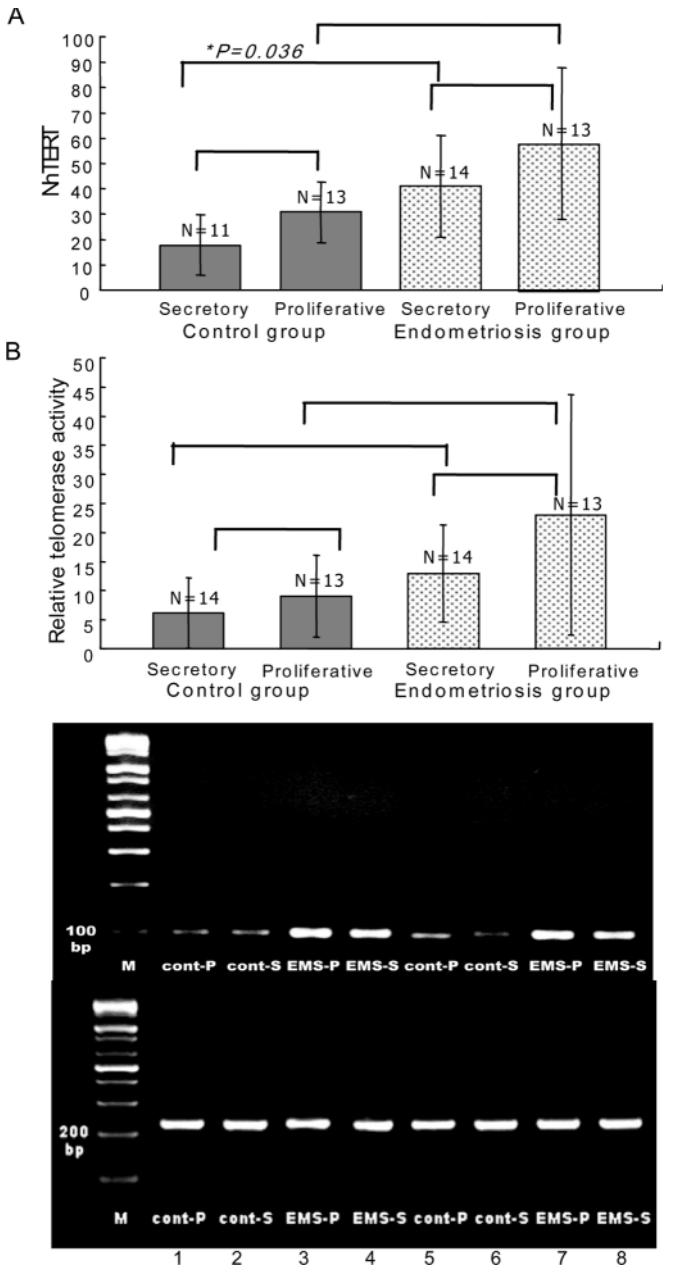

Figure 2. Human telomerase reverse transcriptase (hTERT) mRNA expression and telomerase activity in the study groups according to menstrual phase. (**A**) The hTERT mRNA level during the secretory phase in the control group was compared with the endometriosis group and was found to be significantly higher in the latter (17.7 ± 25.1 versus 47.2 ± 40.1, P = 0.036). Although the hTERT mRNA level during the proliferative phase was higher in the endometriosis group than in the contriol group, the difference was not significant. (**B**) Telomerase activity was higher during the proliferative phase than during the secretory phase for each group and was higher in the endometriosis group than in the control group during each phase, but no significant differences were found. In both groups, all samples without hTERT mRNA and telomerase activity were from the secretory phase. (**C**) The expression pattern of glyceraldehyde-3-phosphatedehydrogenase (GAPDH) (lower) and hTERT mRNA (upper) in the both groups by representative RT–PCR bands. Lanes 1, 5, proliferative phase of control group; lanes 2, 6, secretory phase of control group; lanes 3, 7, proliferative phase of endometriosis group; and lanes 4, 8, secretory phase of endometriosis group. *P < 0.05, N hTERT, normalized hTERT mRNA.

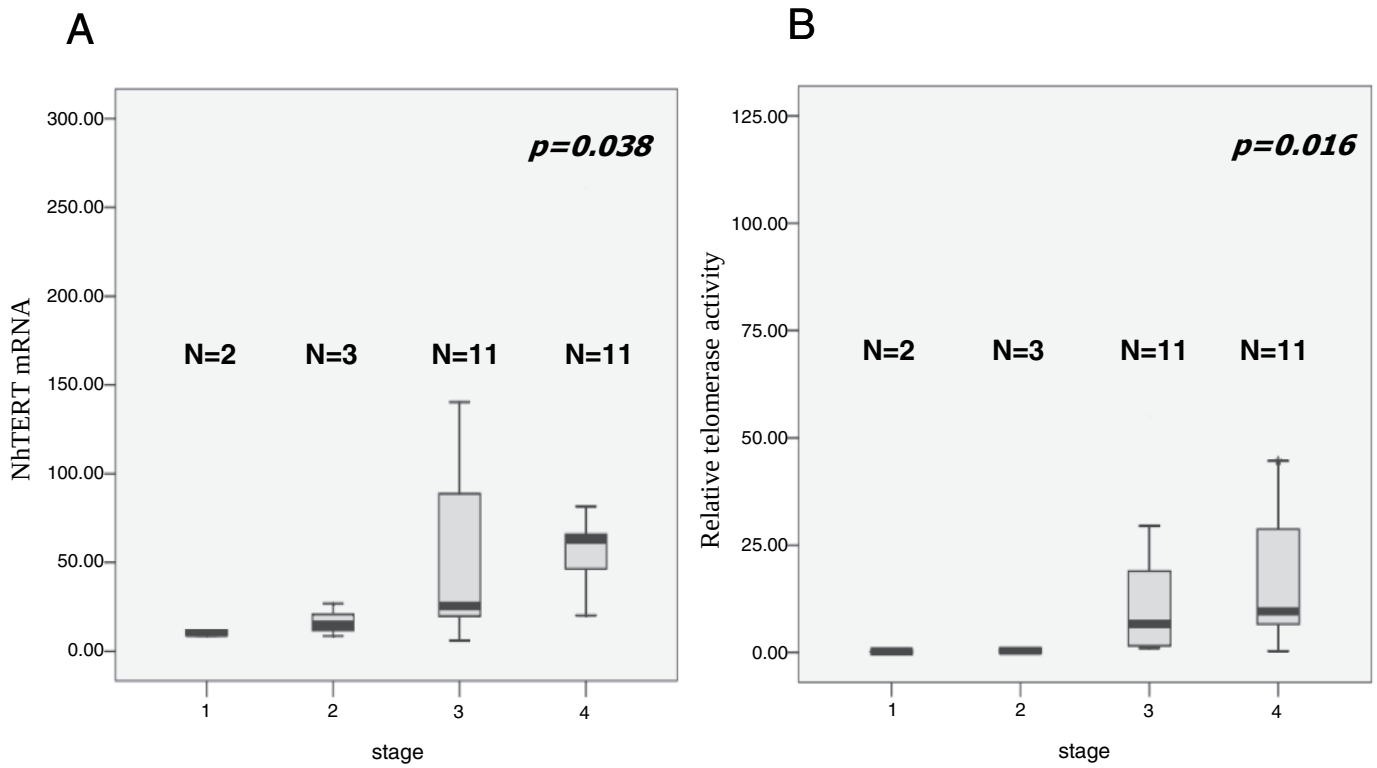

Figure 3. Comparison of human telomerase reverse transcriptase (hTERT) mRNA expression and telomerase activity between clinical disease stages in the endometriosis group. (**A**) hTERT mRNA expression increased significantly as the disease became more severe (P = 0.038). (**B**) A trend of increasing telomerase activity with increasing stage of endometriosis (P = 0.016) was observed. All samples without hTERT mRNA and telomerase activity were those of minimal-to-mild disease (two cases in stage I and one case in stage II).

Table III. Telomerase activity in the study groups

	Control group	Endometriosis	*P* value
Telomerase activity expression(%)	27/30 (90%)	27/30 (90%)	0.122
Relative telomerase activity (RTA)	7.6 ± 13.2	17.8 ± 30.8	
	7.6 ± 13.2	III–IV EMS (n = 22) 21.8 ± 33.0	0.048*

III–IV EMS, moderate-to-severe endometriosis.
Data are expressed as mean ± SD.
*P < 0.05.

As shown in Figure 1B, telomerase activity was revealed to have a pattern similar to the level of hTERT mRNA expression when assessed according to menstrual phase. Six samples without telomerase activity were all from the late-secretory phase. In both the control and the endometriosis groups, telomerase activity was higher in the proliferative phase than in the secretory phase, but no significant differences were found. Also, when the control and endometriosis groups in each menstrual phase were compared, telomerase activity was higher in the latter, although the differences were not significant (Figure 2B).

We analysed the level of RTA according to clinical disease stage in the endometriosis group and found a trend of increasing telomerase activity with increasing stage of endometriosis (Figure 3B). This result was similar to the results of hTERT mRNA and was statistically significant (P = 0.016).

There was no significant correlation between telomerase activity and the size of the endometriotic cyst in the endometriosis group (r = 0.178, P=0.197). Moreover, when the level of telomerase activity and serum CA 125 were compared, no significant correlation was detected (r = 0.199, P = 0.219).

Correlation of hTERT mRNA and telomerase activity

We examined the relationship between hTERT mRNA expression by quantitative real-time RT–PCR and telomerase activity by TRAP assay in all samples. A significant, positive correlation was found, and the levels of hTERT mRNA and telomerase activity showed a linear association (r = 0.778, P < 0.001) (Figure 4).

Discussion

Endometriosis is characterized by the growth of endometrial tissue outside the uterus, but the aetiology and the pathogenesis of this disease remain unclear. Endometriosis tends to recur and progress, and the cumulative recurrence rate is ~40% after 5 years, regardless of medical or surgical treatment (Fedele *et al.*, 1994). Therefore, it is very important to determine the pathogenesis of this disease.

Several studies have focused on the overexpressed genes in the endometrium of endometriosis patients. Persistent expression

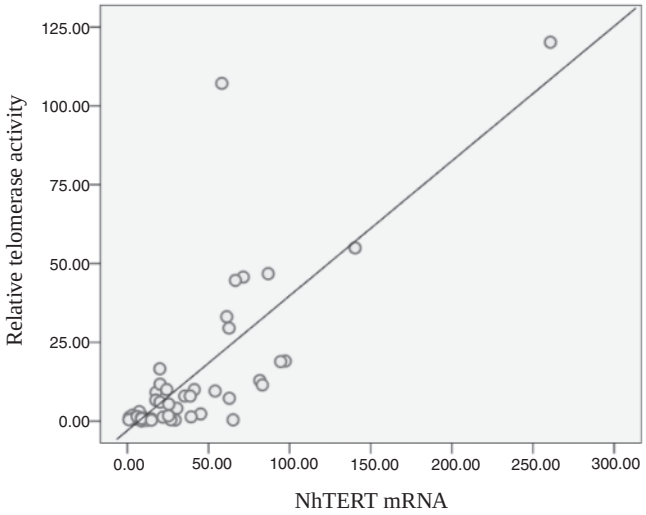

Figure 4. Correlation between human telomerase reverse transcriptase (hTERT) mRNA expression and telomerase activity. A significant, positive correlation was found, and the level of hTERT mRNA and telomerase activity showed a linear association ($r = 0.778$, $P < 0.001$).

of matrix metalloproteinases (MMPs), abundant expression of several angiogenic factors, including interleukins 1, 6 and 8, and increased expression of fibroblast growth factors could contribute to the establishment of endometriotic lesions in the peritoneal cavity (Osteen *et al*., 1999; Taylor *et al*., 2002). Up-regulation of anti-apoptotic genes and down-regulation of pro-apoptotic genes have also been observed in the endometrium of endometriosis patients (Harada *et al*., 2004). Likewise Giudice and Kao (2004) reported that there is an intrinsic abnormality in eutopic endometrium in women with endometriosis that permits these tissues to attach, survive, invade and establish a blood supply in the peritoneun.

In this study, we examined hTERT mRNA expression and telomerase activity to evaluate the limitless replication potential of the endometrium in endometriosis patients. Higher levels of both parameters were detected in endometriosis patients when compared with the normal control group.

The hTERT mRNA was not expressed in 10% (3/30) of the endometriosis group and 20% (6/30) of the control group. These results are similar to previous studies, regarding hTERT mRNA expression in normal endometrium (Kyo *et al*., 1999; Lehner *et al*., 2002). All samples without expression were from the late-secretory phase. Telomerase activity was not detected in 10% of samples from both groups. This expression rate was somewhat higher than that of other studies, because we used a real-time TRAP assay instead of the conventional TRAP assay (Shroyer *et al*., 1997; Maida *et al*., 2002). The conventional TRAP assay requires complex post-amplification procedures, such as polyacrylamide gel electrophoresis, autoradiography and densitometry for measurement of telomerase products, whereas a real-time TRAP assay reduces the risk of carryover contamination and provides a more rapid and reliable quantification of telomerase activity (Wege *et al*., 2003). The samples without telomerase activity were all from the late-secretory phase. Particularly, within the endometriosis group, all samples without telomerase activity were those of minimal-to-mild disease. There may be a number of reasons why the expression was not detected in some women. First, telomerase activity might have been below the threshold of detection of available assay. Also, an alternative mechanism compensating for the end-replication problem could have existed.

Our experiment demonstrated a significant difference in hTERT mRNA levels between the two groups. However, when the level of telomerase activity was compared, we found a prominent difference only between moderate-to-severe endometriosis patients and the control group. These results reflect the presence of multiple mechanisms for regulating telomerase activity. Post-transcriptional regulation of the hTERT gene, such as alternative splicing of hTERT transcripts and protein kinase C-mediated phosphorylation of hTERT, can modulate the enzyme activity at multiple levels (Li *et al*., 1998; Ulaner *et al*., 2000).

In this study, the patterns of hTERT mRNA and telomerase activity, throughout the menstrual phase, were consistent with a previous study (Kyo *et al*., 1999). This suggests that telomerase activation is closely associated with cellular proliferative activity and self-regeneration of the endometrium. In addition, Belair *et al*. (1997) reported that telomerase activity is a biomarker of cell proliferation, rather than that of malignant transformation. Estrogen may play a role in the regulation of telomerase activity as a potent inducer of endometrial proliferation. Telomerase expression, in fact, declines rapidly after ovulation when the estrogen level decreases. Furthermore, it is suppressed by progesterone (Williams *et al*., 2001). The interesting feature of this study was that the hTERT mRNA level during both the proliferative and secretory phases is higher in the endometriosis group when compared with that in the control group, although the difference was only significant for the secretory phase. This may indicate that the tissue from endometriosis is different from normal endometrium, which undergoes estrogen-induced cell proliferation that is suppressed by progesterone.

We found that hTERT mRNA expression and telomerase activity were significantly different according to the different stages of endometriosis. This finding suggests that hTERT mRNA expression and telomerase activity may reflect the severity of the disease. However, we had a small number of samples with the minimal-to-mild disease. Therefore, further studies with a large number of cases are needed to confirm this result.

This study revealed that there was a statistically significant association between hTERT mRNA level and telomerase activity in both groups. However, we found discordant results in some cases: a few patients had relatively high telomerase activity with a low hTERT mRNA level, whereas some cases demonstrated the inverse association. The former may result from inefficient RNA extraction or RNA degradation in the clinical samples, whereas the latter may reflect suboptimal extraction of telomerase or enzyme instability. In addition, other mechanisms, such as post-transcriptional regulation, may play an important role in telomerase activity.

In conclusion, our results represent the first evidence that telomerase activity and hTERT mRNA expression are

correlated with the proliferative potential of the endometrium in endometriosis patients. In addition, we suggest that the replication potential of regurgitated endometrial tissue in the peritoneal cavity is crucial for the development of viable implants and may have an important role in the pathogenesis of endometriosis.

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