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The roles of thioredoxin and thioredoxin-binding protein-2 in endometriosis

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BACKGROUND: Oxidative stress is considered to be involved in the establishment and development of endometriosis. Thioredoxin (TRX) is an endogenous redox regulator that protects cells against oxidative stress, and TRX-binding protein-2 (TBP-2) is a negative regulator of TRX in the biological function and expression. The aim of this study was to investigate the roles of TRX and TBP-2 in the pathophysiology of endometriosis.

METHODS: A total of 35 patients with histologically confirmed endometriosis and 31 patients without endometriosis participated in this study. Real-time polymerase chain reaction was used to quantify TRX and TBP-2 mRNA levels, and immunohistochemistry (IHC) was used to assess TRX and TBP-2 protein localization in the endometrium. Serum and peritoneal fluid levels of TRX and TBP-2 were measured using a specific commercial ELISA.

RESULTS: There were no significant differences in TRX mRNA levels in the endometrium of patients with endometriosis and the control groups. However, TBP-2 mRNA levels in the endometrium were lower, and the TRX to TBP-2 ratio was higher in patients with endometriosis than in the control group. In particular, the TRX to TBP-2 ratio was significantly higher during late secretory and menstrual phase in patients with endometriosis compared with the control group. IHC studies also showed the decreased TBP-2 immunoreactivity in patients with endometriosis compared with the control group. There was no correlation between TRX and TBP-2 mRNA levels in patients with endometriosis, whereas TRX mRNA levels were positively correlated with TBP-2 mRNA levels in the control group. There were no significant differences between the two groups in TRX and TBP-2 levels in serum or peritoneal fluid.

CONCLUSIONS: Aberrant expression of TRX and TBP-2 in the endometrium may be associated with the establishment of endometriosis.

Key words: oxidative stress / endometriosis / thioredoxin / thioredoxin-binding protein-2

Introduction

Endometriosis, or the growth of hormone-responsive endometrial glands and stroma outside of the uterus, is a chronic pleomorphic condition responsible for infertility and pelvic pain. It is a common disease, reported to occur in around 10% of women of reproductive age and in 30–50% of infertile women (Cramer and Missmer, 2002; Pritts and Taylor, 2003). Various theories have been proposed to explain the pathogenesis of endometriosis, but its overall etiology has not yet been established.

Oxidative stress is associated with numerous pathological conditions including aging, cardiovascular disease, neurodegenerative disease, and cancer. Oxidative stress is also considered to be one of the most important factors affecting the establishment and development of endometriosis (Jackson *et al.*, 2005; Agarwal *et al.*, 2006). Many previous studies have reported that levels of some oxidative stress and antioxidant biomarkers found in serum or peritoneal fluid are significantly different between patients with and without endometriosis (Ho *et al.*, 1997; Jackson *et al.*, 2005; Verit *et al.*, 2008; Lambrinoudaki *et al.*, 2009). Additionally, it has been reported that there is no cyclic variation of antioxidant enzymes in the endometrium of patients with endometriosis (Ota *et al.*, 1999). However, these studies varied in their method, and their results are not consistent. Therefore, the association between oxidative stress and endometriosis is still a matter of debate.

Thioredoxin (TRX) is a redox-regulating antioxidant protein that prevents cell damage from oxidative stress (Nordberg and Arner,

2001). TRX exhibits various biological activities in cellular processes such as cell proliferation and apoptosis. TRX also controls many transcription factors such as AP-1 (Abate et al., 1990), NF-κB (Qin et al., 1994) and p53 (Ueno et al., 1999). TRX is linked to increased tumor cell growth and inhibited apoptosis and has been reported to be associated with various human cancers (Kaimul et al., 2007). In addition, TRX has been demonstrated in endometrium, decidua, trophoblast and fetal tissue, where TRX may play an important role in blastocyst implantation and fetal growth (Maruyama et al., 1997; Kobayashi-Miura et al., 2007).

TRX-binding protein-2 (TBP-2), also known as TRX-interacting protein or vitamin D_3 up-regulated protein, regulates the expression and function of TRX (Nishiyama et al., 1999; Junn et al., 2000). Recent studies have elucidated the role of TBP-2 in biologically important cellular events. Accumulated evidence suggests that increased expression of TBP-2 is related to growth suppression and apoptosis promotion in cells pre-exposed to oxidation through blocking TRX activity. Indeed, the down-regulation of TBP-2 has been observed in several human cancers (Kaimul et al., 2007).

These findings support the hypothesis that TRX and TBP-2 are involved in the protection of cells against oxidative stresses and the regulation of cellular proliferation in a number of tissues, and consequently, that dysregulation of TRX and TBP-2 is associated with a number of disease processes, including those of the reproductive organs. However, few studies have been reported regarding the expression and function of TRX and TBP-2 in the human endometrium. In addition, there have been no reports about the roles of TRX and TBP-2 in the development of endometriosis.

This study was conducted to compare the TRX and TBP-2 expression of endometrium between the endometriosis and control group. We also evaluated the TRX and TBP-2 levels of serum and peritoneal fluid in patients with and without endometriosis.

Materials and Methods

Participants

Patients aged 20–45 years who were being managed for benign gynecologic diseases at the Gangnam Severance Hospital, Seoul, Korea, participated in this study. All participants had regular menstrual cycles and none of them had received hormonal treatments, such as GnRH agonists or oral contraceptives, for at least 3 months prior to surgery. Patients with diseases closely associated with oxidative stress, such as cancer or cardiovascular disease, or who had taken antioxidants, were excluded. We also excluded patients with endometrial pathology including endometrial polyps and endometrial hyperplasia.

Endometrial tissues were obtained by curettage before surgical procedures and used for real-time polymerase chain reaction (PCR) and immunohistochemistry (IHC). For those patients, blood and peritoneal fluid samples were also collected. The diagnosis of endometriosis was confirmed by pathological examination and the clinical stage of the disease was classified in accordance with the American Society for Reproductive Medicine (ASRM) revised American Fertility Society (AFS) scoring system. We diagnosed minimal to mild (Stages I and II) endometriosis in eight patients and moderate to severe (Stages III and IV) endometriosis in 27 patients. The control group consisted of 18 patients with benign ovarian cysts, 10 patients with cervical intraepithelial neoplasia, and three patients without visible pathology in the pelvis. Benign ovarian cysts included simple cysts, dermoid cysts, serous cystadenoma and mucinous cystadenoma.

The classification of endometrium was determined according to the last menstrual period and histology of endometrium (Noyes et al., 1950) and divided into six phases of the menstrual cycle (menstrual, early, late proliferative and early-, mid- or late-secretory phase). Thirty-five women with endometriosis were from the menstrual ($n = 5$), early proliferative ($n = 7$), late proliferative ($n = 6$), early-secretory phase ($n = 6$), mid-secretory phase ($n = 6$) and late-secretory phase ($n = 5$). Thirty-one control women were from the menstrual ($n = 5$), early proliferative ($n = 5$), late proliferative ($n = 6$), early-secretory phase ($n = 5$), mid-secretory phase ($n = 5$) and late-secretory phase ($n = 5$).

The institutional review board of the Gangnam Severance Hospital approved this study, and written consent was obtained from all participants.

RNA extraction and real-time PCR

Total RNA was extracted from endometrial samples using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). According to the manufacturer's protocols, reverse transcription into cDNA was carried out from 2 μg of total RNA from each sample using SuperScript™ III first-strand synthesis system (Invitrogen, Carlsbad, CA, USA). TRX and TBP-2 mRNA levels were measured through a SYBR green real-time PCR using the ABI PRISM 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a housekeeping gene, was used as a normalization control. Specific primers for TRX (primer: 5′-AGCAGATCGAGAGCAAGACT-3′, reverse primer: 5′-CTCTGAAGCAACATCCTGAC-3′) and TBP-2 (forward primer: 5′-ATAGCGCAGGTACTCCGAAG-3′, reverse primer: 5′-GGTGAAGTGGAGGTGTGTG-3′) were designed. The sequences of forward and reverse primer for GAPDH were 5′-GAAGGTGAAGG TCGGAGTC-3′ and 5′-GAAGATGGTGATGGGATTTC-3′. Real-time PCR was performed in a 20-μl buffer including 2 μl of cDNA, 5 pM of each primer and power SYBR green PCR master mix (Applied Biosystems). The thermal cycling conditions were 50°C pre-incubation for 2 min and 95°C denaturation for 10 min followed by 40 cycles of 95°C denaturation for 15 s and 60°C annealing/extension for 1 min. Relative TRX and TBP-2 mRNA levels were calculated by the ΔΔCt method as described previously (Livak and Schmittgen, 2001). The normalization formula target amount was $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = [Ct \text{ (candidate gene)} - Ct \text{ (GAPDH gene)}] - [Ct \text{ (candidate gene calibrator)} - Ct \text{ (GAPDH gene calibrator)}]$.

Immunohistochemistry

Paraffin-embedded sections were deparaffinized with xylene, rehydrated in a graded series of ethanol, and immersed in 3% H_2O_2 for 10 min to block endogenous peroxidase activity. Sections were incubated with TRX and TBP-2 primary antibody (1:200 dilution; Upstate, Lake Placid, NY, USA) overnight at room temperature and then incubated with a biotin-free polymeric horseradish peroxidase-linked antibody conjugate system (LabVision, Fremont, CA, USA) for 30 min at room temperature. The chromogen was developed for 2 min. Slides were counterstained with hematoxylin and mounted in Immunomount for examination. A negative control was prepared by applying a secondary antibody without a primary antibody. Representative micrographs of the IHC were photographed by a Nikon DXM1200F digital camera (Nikon, Japan) at ×400 magnifications with 3840 × 3072 pixel resolution.

The IHC intensity for TRX and TBP-2 was assessed using the following intensity categories: 0 (no staining), 1+ (weak staining), 2+ (moderate staining) and 3+ (strong staining). For each slide, a *H*-score was calculated as follows: *H*-score = (% of cells that stained at intensity category 1 × 1) + (% of cells that stained at intensity category 2 × 2) + (% of cells that stained at intensity category 3 × 3). Slides were scored at different

times by two investigators blinded to any clinical data. The average score of the two was used for the final analysis.

Measurements of TRX and TBP-2 protein levels in serum and peritoneal fluid

Blood samples were collected in sterile tubes before surgery and centrifuged at 300*g* for 10 min. Serum samples were obtained and stored at −80°C. Peritoneal fluid samples were aspirated during laparoscopy from cul-de-sac and obtained from 20 subjects in each group. Peritoneal fluid samples were also centrifuged at 300*g* for 10 min and stored at −80°C. Serum and peritoneal fluid levels of TRX and TBP-2 were determined by an ELISA using a commercial kit (USCNLIFE, Missouri City, TX, USA) according to the manufacturer's protocols. The minimum detectable concentration was 0.78 ng/ml for TRX and 0.156 ng/ml for TBP-2.

Statistical analysis

Data were expressed as mean ± SD or mean ± SEM where appropriate. Student's *t*-test was used to compare the clinical and laboratory characteristics of the endometriosis and control groups. The Mann–Whitney *U*-test was used to compare the mRNA levels and H-scores of TRX and TBP-2 between the two groups. Serum and peritoneal fluid levels of TRX and TBP-2 were also compared using the Mann–Whitney *U*-test. The differences in the mRNA expression and IHC staining scores of TRX and TBP-2 through the menstrual cycle were compared using the Kruskal–Wallis test followed by the Dunn procedure within each group. Pearson's correlation coefficient analysis was used for the evaluation of the correlation between the TRX and TBP-2 mRNA levels in the endometrium. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 15.0 software (SPSS Inc., Chicago, IL, USA). *P*-values of <0.05 were considered to be statistically significant.

Results

TRX and TBP-2 mRNA levels in the endometrium

TRX mRNA levels were not significantly different between individuals with or without endometriosis. However, the TBP-2 mRNA levels were significantly lower in the endometriosis group, and consequently the TRX to TBP-2 ratio was significantly higher in patients with endometriosis than in the control group (Fig. 1).

There was no variation in TRX and TBP-2 mRNA levels within either group during the menstrual cycle. TRX mRNA levels were not significantly different between the two groups during the whole of the menstrual cycle. However, the TBP-2 mRNA levels were significantly lower in patients with endometriosis than in the control group during the menstrual phase. TBP-2 mRNA levels were also considerably lower in patients with endometriosis during the late secretory phase, although the differences were not statistically significant ($P = 0.076$). The TRX to TBP-2 ratio was significantly higher in patients with endometriosis than in the control group during the late-secretory and menstrual phases (Table I and Fig. 2).

Relationships between TRX and TBP-2 mRNA levels in the endometrium

Pearson's correlation coefficient was used to investigate the interaction between TRX and TBP-2 mRNA levels. TRX mRNA levels positively correlated with the TBP-2 mRNA levels in the control group. However, there was no correlation between TRX and TBP-2 mRNA levels in the endometriosis group (Fig. 3).

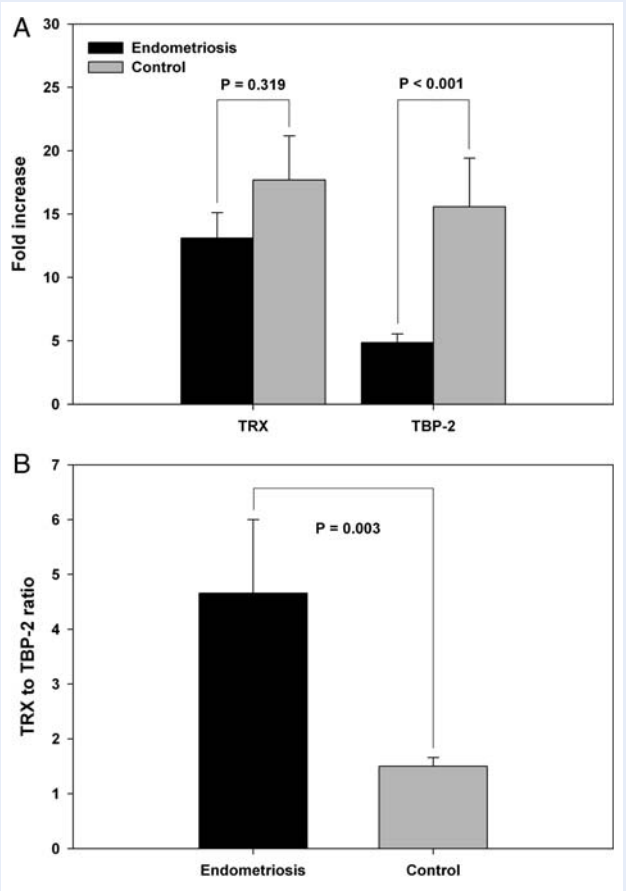

Figure 1 TRX and TBP-2 mRNA levels in the endometrium of endometriosis and control group (**A**) and comparison of the TRX to TBP-2 ratio between the two groups (**B**). Data are expressed as mean ± SEM.

Immunohistochemistry

The expression of TRX and TBP-2 was observed in endometrial glandular and stromal cells throughout the menstrual cycle (Fig. 4). The *H*-scores of TRX were not significantly different between the two groups. However, the *H*-scores of TBP-2 were significantly lower in patients with endometriosis than in the control group (Fig. 5A).

There was no variation in the *H*-scores of TRX and TBP-2 in either group during the menstrual cycle. When the *H*-scores of TRX and TBP-2 were compared according to the menstrual cycle within each group, *H*-scores of TRX were significantly higher than those of TBP-2 during the late-secretory and menstrual phases in patients with endometriosis (Fig. 5B and C).

Serum and peritoneal levels of TRX and TBP-2

TRX and TBP-2 levels in the serum and peritoneal fluid were not significantly different between the two groups. In both groups, the TRX

Table I Comparison of the TRX and TBP-2 mRNA levels in the endometrium through the menstrual cycle.

	M	EP	LP	ES	MS	LS	*P*-value[a]
TRX							
Endometriosis	27.06 ± 7.96	5.65 ± 1.60	10.17 ± 3.63	12.62 ± 2.49	9.47 ± 3.02	17.99 ± 6.89	0.102
Control	6.84 ± 1.72	21.17 ± 8.20	15.34 ± 5.27	30.56 ± 17.88	15.15 ± 2.50	17.59 ± 6.99	0.267
P-value[b]	0.076	0.123	0.423	0.465	0.201	0.754	
TBP-2							
Endometriosis	1.95 ± 0.58	3.33 ± 0.95	4.06 ± 1.20	7.67 ± 1.83	7.79 ± 2.33	3.99 ± 1.26	0.265
Control	9.59 ± 3.77	8.50 ± 2.50	8.34 ± 2.94	30.71 ± 18.43	20.46 ± 12.65	17.31 ± 7.24	0.562
P-value[b]	0.016	0.167	0.262	0.361	0.855	0.076	
TRX/TBP-2							
Endometriosis	18.42 ± 6.87[c]	1.99 ± 0.46	2.37 ± 0.59	2.05 ± 1.06	1.30 ± 0.18	4.59 ± 1.23[c]	0.004
Control	0.88 ± 0.17	2.25 ± 0.50	1.90 ± 0.31	1.33 ± 0.36	1.45 ± 0.37	1.07 ± 0.28	0.074
P-value[b]	0.008	0.935	0.423	0.201	0.715	0.009	

Values are expressed as mean ± SEM. M, menstrual phase; EP, early-proliferative phase; LP, late-proliferative phase; ES, early-secretory phase; MS, mid-secretory phase; LS, late-secretory phase.
[a]Kruskal–Wallis test was used to analyze the differences of the TRX or TBP-2 mRNA levels in the endometrium through the menstrual cycle within each group.
[b]Mann–Whitney *U*-test was used to analyze the differences of the TRX and TBP-2 mRNA levels in the endometrium between two groups.
[c]Statistically significant versus MS in Dunn procedure.

levels were higher than the TBP-2 levels in the serum, but the TRX levels were not different from the TBP-2 levels in the peritoneal fluid (Table II).

Discussion

In this study, we have demonstrated a strong correlation between TRX and TBP-2 mRNA levels in normal endometrium. We have also demonstrated that altered TRX and TBP-2 expressions in the endometrium are associated with the development of endometriosis. Furthermore, we investigated the levels of TRX and TBP-2 in the serum and peritoneal fluid.

When comparing patients with endometriosis to a control group regardless of the phase of the menstrual cycle, we did not find any differences in TRX mRNA levels in endometrium. However, we discovered that the levels of TBP-2 mRNA were significantly lower ($P < 0.001$), and the rate of TRX/TBP-2 was significantly higher ($P = 0.003$) in patients with endometriosis. In addition, we also observed decreased TBP-2 immunoreactivity in patients with endometriosis compared with the control group ($P < 0.001$). On the other hand, the control group showed a mutually significant correlation between the TRX and the TBP-2 mRNA levels in endometrium ($r = 0.793$, $P < 0.001$), but patients with endometriosis did not show a significant correlation. On the basis of these results, we may infer that mutual disproportion of TRX and TBP-2 influenced by oxidative stress occurs in patients with endometriosis, which may be due to decreased expression of TBP-2, a negative regulator of TRX, rather than increased expression of TRX. Down-regulation of TBP-2 expression has been reported in numerous human cancers including prostate cancer, bladder cancer, breast cancer and colon cancer (Kaimul *et al.*, 2007). Loss of TBP-2 has also been associated with poor prognosis in patients with B-cell lymphoma (Tome *et al.*, 2005). TBP-2 expression up-regulation by histone deacetylase inhibitors, known to be a specific class of anticancer agents, simultaneously occurred with a significantly decreased expression of TRX (Butler *et al.*, 2002). Therefore, it seems that TBP-2 is one of the potent antitumor genes active in growth regulation and plays a crucial role in the development of endometriosis.

When we compared the mRNA levels of TRX and TBP-2 based on the menstrual cycle between the two groups, statistical significance was not found in either the proliferative or early-secretory phases. However, TBP-2 mRNA levels were lower and the rate of TRX/TBP-2 was higher in the endometriosis group than in the control group during late-secretory and menstrual phases. Consequently, compared with the control group, patients with endometriosis showed relatively higher TRX activity resulting from down-regulated TBP-2 during the late-secretory and menstrual phases in comparison with other phases of the menstrual cycle. Apoptosis fosters cell homeostasis in the human endometrium. It is believed that the viability of regurgitated endometrial cells is facilitated by a decrease in the normal apoptosis process and an increase in cellular proliferation (Harada *et al.*, 2004). These cells are then implanted in other sites outside the uterus, which causes endometriosis. In patients with endometriosis, it has been reported that apoptosis is significantly decreased in the endometrium during the late-secretory and menstrual phases, and the endometrium shows higher proliferation activity, clinically as well as pathologically. Increased TRX levels are positively correlated with cell proliferation and negatively correlated with apoptosis in human gastric carcinoma (Grogan *et al.*, 2000). TRX levels are also increased in several human cancers such as cervical carcinoma, lung cancer and pancreatic cancer (Kaimul *et al.*, 2007). Therefore, it may be presumed that the increased TRX activity in endometrial tissues during the late-secretory and menstrual phases plays an important role in the development of endometriosis through adhesion, infiltration and

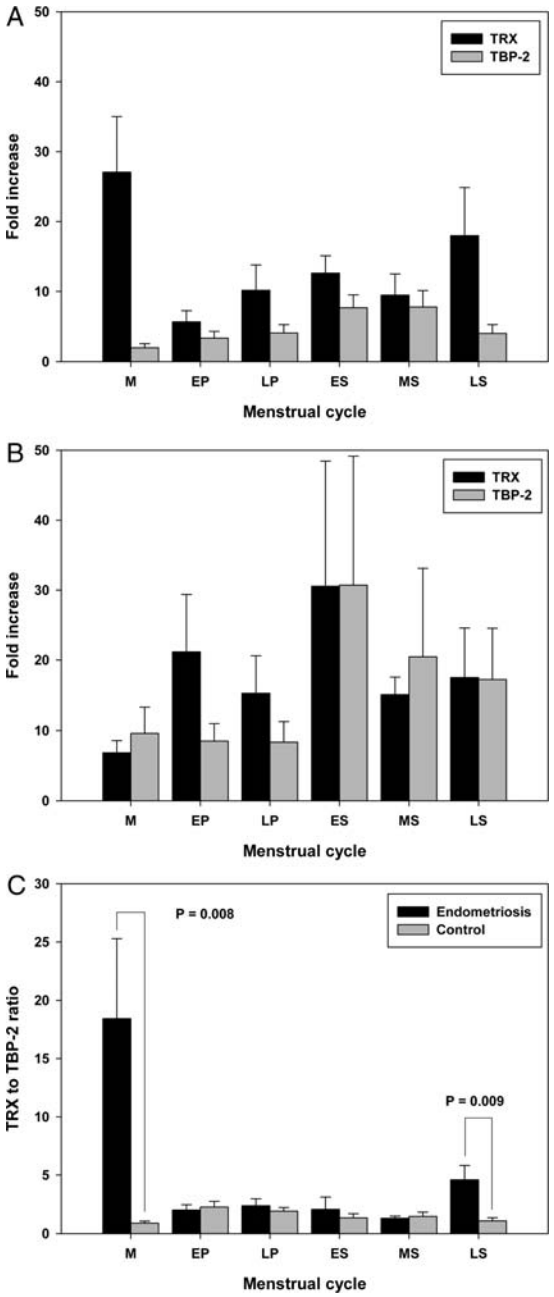

Figure 2 TRX and TBP-2 mRNA levels in the endometrium of the endometriosis (**A**) and control group (**B**) and a comparison of the TRX to TBP-2 ratio between the two groups (**C**) throughout the menstrual cycle. M, menstrual phase; EP, early-proliferative phase; LP, late-proliferative phase; ES, early-secretory phase; MS, mid-secretory phase; LS, late-secretory phase. Data are expressed as mean ± SEM.

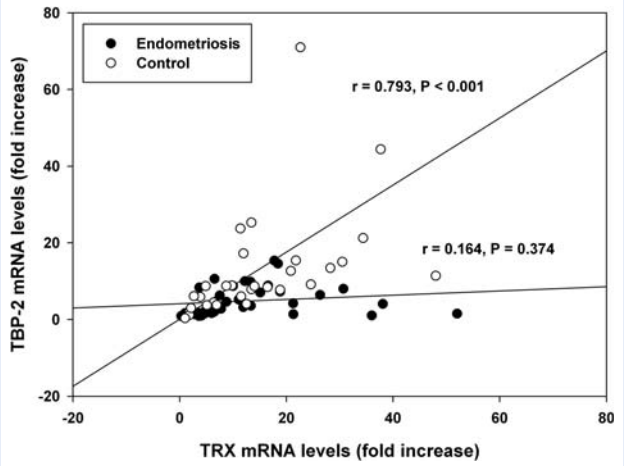

Figure 3 Pearson's correlation coefficients between TRX and TBP-2 mRNA levels in the endometriosis and control group.

proliferation of endometrial cells regurgitated into the peritoneal cavity by enhancing endometrial cell growth and suppressing apoptosis.

Previous reports demonstrated that the expression of TRX increased during the early-secretory phase of the menstrual cycle and that it modulates endometrial cell growth, which is regulated by estrogen and progesterone (Maruyama *et al*., 1997, 1999). It has also been reported that, in mice, the TRX knockout is lethal during the early post-implantation period (Matsui *et al*., 1996). These results imply that the expression of TRX in the endometrium is closely linked to estrogen and progesterone levels and may play an important role in blastocyst implantation as well as normal embryo development.

One of the most important findings of our study was that TRX mRNA levels were significantly correlated with TBP-2 mRNA levels in the normal endometrium. Consequently, it appears that TBP-2 expression in the endometrium is influenced by estrogen and that TBP-2 may influence implantation of blastocyst and the development of the embryo. However, in our study, we found no statistically significant differences in TRX expression during the menstrual cycle, although both TRX and TBP-2 expression increased somewhat at the early-secretory phase compared with the other menstrual phases. An earlier study using immunostaining also did not demonstrate a significant increase in TRX expression at the early-secretory phase, while TRX expression at the secretory phase was relatively higher than at the proliferative phase (Stavreus-Evers *et al*., 2002).

Several studies have regarded oxidative stress as an important cause of infertility in endometriosis. These studies found that levels of nitrogen oxide and superoxide dismutase change during the menstrual cycle and are highly expressed at the secretory stage; however, these changes were not found in patients with endometriosis (Ota *et al*., 1999). Other antioxidants, such as xanthine oxidase and catalase, also show abnormal expression in the endometrial tissue of patients with endometriosis (Ota *et al*., 2002). The present study demonstrated that patients with endometriosis had marked alterations in the expression of TRX and TBP-2 according to the menstrual cycle. These facts considered, and it may be concluded that antioxidants show differences in their expression in endometrial tissues according to the menstrual cycle and that these differences are regulated by an ovarian steroid hormone. When the balance between the production of reactive oxygen species and antioxidant defense system is

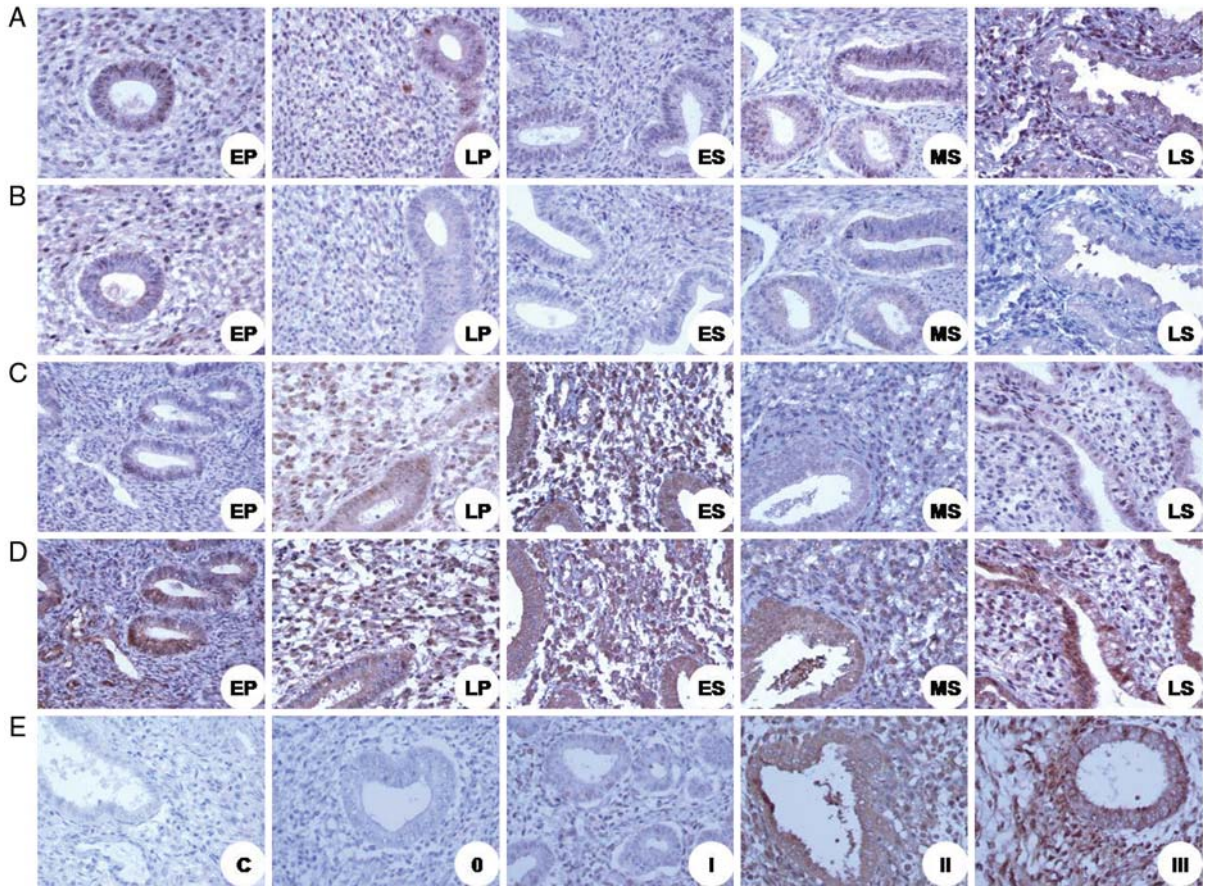

Figure 4 Expression of TRX and TBP-2 in the endometrium (×400). Representative micrographs of TRX (**A**) and TBP-2 (**B**) expression of the endometriosis group, and TRX (**C**) and TBP-2 (**D**) expression of the control group in endometrium throughout the menstrual cycle. (**E**) Negative control and the staining intensity of IHC. EP, early-proliferative phase; LP, late-proliferative phase; ES, early-secretory phase; MS, mid-secretory phase; LS, late-secretory phase; C, negative control.

disrupted, the possibility of endometriosis increases and it may thus be connected to infertility.

The levels of TRX and TBP-2 protein in serum and peritoneal fluid did not show a difference in either group, and there was no correlation with the mRNA expression in the endometrium. These results indicate that the changes in TRX and TBP-2 expression in the endometrium do not lead to changes in serum and peritoneal fluid levels and that the levels of TRX and TBP-2 in the serum and peritoneal fluid have no relation to the development of endometriosis. Our results corresponded with those of earlier studies that showed that the levels of TRX in serum had no relation to endometriosis (Lambrinoudaki *et al.*, 2009). It is presumed that alteration of TRX and TBP-2 expression in the endometrium may facilitate the inflammatory process to induce the development of endometriosis by activating a transcription factor such as NF-κB, which is associated with the recruitment of macrophages and the secretion of inflammatory cytokines. Moreover, it is known that the serum TRX level is affected by various conditions, such as rheumatoid arthritis (Yoshida *et al.*, 1999), type 2 diabetes (Kakisaka *et al.*, 2002) and nonalcoholic fatty liver disease (Sumida *et al.*, 2003). Accordingly, alteration of TRX and TBP-2 expression in the endometrium may not be reflected in the serum and peritoneal fluid levels of TRX and TBP-2.

To our knowledge, this report is the first to investigate the influences of oxidative stress on the development of endometriosis by comparing the mRNA levels of TRX and TBP-2 in the endometrium of patients with endometriosis to those of a control group. TBP-2 expression and the relationship between TRX and TBP-2 expression in normal human endometrium were also reported in this study.

This study does have some limitations. We did not have sufficient participants to compare TRX and TBP-2 levels from individuals with minimal and mild endometriosis to the levels found in those with moderate and severe endometriosis. Additionally, when subjects were categorized by the phase of the menstrual cycle, each group included only 5–7 subjects. Thus, we believe that further studies with larger sample sizes are necessary.

In conclusion, TRX mRNA levels significantly correlated with TBP-2 mRNA levels, a negative regulator of TRX function, in the normal endometrium. However, TRX and TBP-2 mRNA levels did not correlate in women with endometriosis. In particular, TBP-2 mRNA levels were lower and the TRX to TBP-2 ratio was higher in patients with

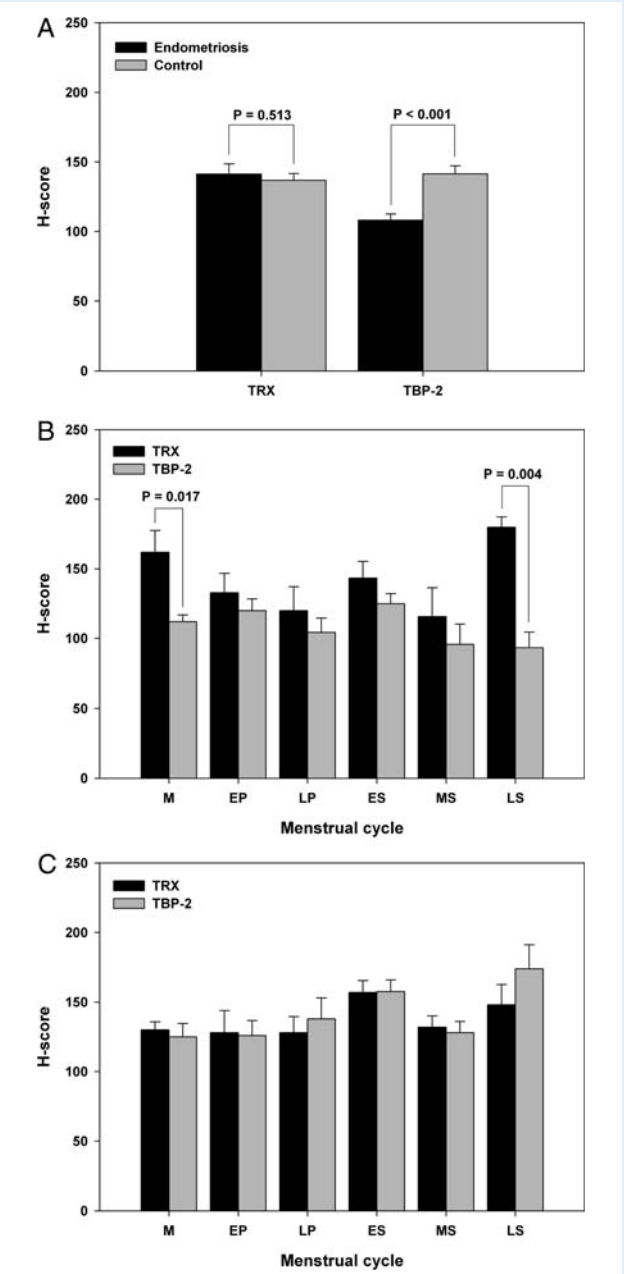

Figure 5 Comparison of the *H*-score of TRX and TBP-2 between the endometriosis and control group (**A**) and *H*-score of TRX and TBP-2 in the endometriosis group (**B**) and control group (**C**) throughout the menstrual cycle. M, menstrual phase; EP, early-proliferative phase; LP, late-proliferative phase; ES, early-secretory phase; MS, mid-secretory phase; LS, late-secretory phase. Data are expressed as mean ± SEM.

endometriosis during the late-secretory and menstrual phases. These results suggest that TRX and TBP play a crucial role in the homeostasis of the normal endometrium and that altered TRX and TBP-2 expression in the endometrium is associated with the establishment of endometriosis. Therefore, TRX and its negative regulator may represent important future targets to develop clinical therapies for endometriosis.

Table II Comparison of the serum and peritoneal fluid levels of the TRX and TBP-2.

	Endometriosis group ($n = 35$)	Control group ($n = 31$)	*P*-value
TRX in serum (ng/ml)	667.0 ± 109.1	716.4 ± 150.0	0.191
TBP-2 in serum (ng/ml)	213.6 ± 63.6	213.3 ± 73.1	0.958
TRX in peritoneal fluid (ng/ml)[a]	349.7 ± 260.5	390.2 ± 189.1	0.488
TBP-2 in peritoneal fluid (ng/ml)[a]	461.5 ± 77.3	459.3 ± 104.5	0.260

Values are expressed as mean ± SEM.
[a]$n = 20$.

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