

Peroxisome proliferator-activated receptor γ agonist suppresses human telomerase reverse transcriptase expression and aromatase activity in eutopic endometrial stromal cells from endometriosis

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Objective: To investigate the effect of peroxisome proliferator activated receptor γ (PPAR γ) agonist on the cell proliferation properties and expression of human telomerase reverse transcriptase (hTERT) and aromatase in cultured endometrial stromal cell (ESC) from patients with endometriosis.

Methods: Human endometrial tissues were obtained from women with endometriosis and healthy women (controls) using endometrial biopsy. Isolated ESCs were cultured and the cell proliferation was measured by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay and expression of hTERT, aromatase, and cyclooxygenase (COX)-2 by western blotting according to the addition of rosiglitazone (PPAR γ agonist).

Results: We demonstrate that the cultured ESCs of endometriosis showed hTERT protein overexpression and increased cellular proliferation, which was inhibited by rosiglitazone, in a dose-dependent manner. At the same time, PPAR γ agonist also inhibited aromatase and COX-2 expression, resulting in decreased prostaglandin E₂ production in the ESCs of endometriosis.

Conclusion: This study suggests that PPAR γ agonist plays an inhibitory role in the proliferative properties of eutopic endometrium with endometriosis by down-regulation of hTERT and COX-2 expression; this could be a new treatment target for endometriosis.

Keywords: Endometriosis; Peroxisome proliferator activated receptor γ ; Rosiglitazone; Human telomerase reverse transcriptase; Aromatase; Endometrium

Introduction

Endometriosis, defined as the presence of endometrial glandular

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and stromal cells in sites outside the uterine cavity, is an estrogen-dependent inflammatory disease. It affects 5% to 10% of women of reproductive age, and the main clinical features are chronic pelvic pain, dyspareunia, and infertility [1]. Although the pathogenesis of endometriosis has not been elucidated, endometriosis is a highly proliferative and benign gynecologic disorder with the ability to undergo neoplastic-like processes. Endometriotic lesions have aberrant overexpression of anti-apoptotic genes, such as bcl-2 [2], various cellular adhesion molecules, and metalloproteinases [3] and low expression of pro-apoptotic genes, such as bax [4]. In addition, molecular distinctions, such as the overproduction of estrogen, prostaglandins [5], and cytokines [6], also occur in the endometrium of women with endometriosis relative to the endometrium of women without

the disease. These conditions are able to enhance the adhesion of shed endometrial tissue onto the ectopic site and the progression of disease.

Endometriosis shares a predisposing factor and molecular similarity with cancer [7]. Increased telomerase activity has been detected in various cancers, such as endometrial and ovarian cancer [8,9], and is also related to a sustained replication potential in endometriosis [10]. Telomeres are the non-coding tandemly repeated DNA sequences (5-TTAGGG-3) that protect all eukaryotic chromosomal ends. They are markers for cellular aging, as their length declines with each cell division. Most human cancer cells maintain their telomeres through activation of telomerase. Telomerase is a ribonucleoprotein complex and plays an important role in telomere maintenance and cellular immortality, and its core functional component is human telomerase reverse transcriptase (hTERT). A correlation between hTERT mRNA expression and telomerase activity has been observed [11]. An immunohistochemical study has suggested that aberrant endometrial expression of telomerase enhances endometrial cell proliferation, contributing to the pathogenesis of endometriosis [12].

Peroxisome proliferator activated receptor γ (PPAR γ) is a pleiotropic nuclear hormone receptor that binds to specific DNA response elements and may regulate gene expression indirectly, negatively, or positively, through competition with other transcription factors [13]. Using a DNA array containing 96 genes known to control the cell cycle, PPAR γ inhibited expression of genes encoding the minichromosome maintenance proteins (MCM) 6 and 7 [14]. MCM proteins play a central role in the regulation of the initiation of DNA replication and ensure that DNA replicates only once during each cell cycle [15]. Therefore, PPAR γ itself is considered to be a novel important regulator of the cell cycle and apoptosis and may provide a new therapeutic approach to the treatment of proliferative cardiovascular diseases and cancer [16-18]. Several reports have shown that PPAR γ agonist (rosiglitazone) treatment induced not only growth inhibition of endometrial explants [19] but also regression of established lesions [20]. We previously reported that rosiglitazone inhibited the phosphorylation of Smad and extracellular signal-regulated kinase (ERK) in human endometrial stromal cell (ESC) decidualization that is induced by TGF- β 1 *in vitro* [21]. During this process, rosiglitazone was able to inhibit PGE $_2$ production through the down-regulation of the ERK pathway. Local positive feedback loops of estrogen and cytokines are essential for ESC proliferation in eutopic endometrium with endometriosis.

Based on the above considerations, we have examined the effects of PPAR γ activation by rosiglitazone on the expression of hTERT, a core functional component of telomerase activity, and on the local cytokine production, prostaglandin E $_2$ (PGE $_2$) pathway through ERK phosphorylation in *in vitro* cultured eutopic ESCs from patients with endometriosis.

Methods

1. Clinical subjects and endometrial biopsies

Human endometrial tissues were obtained from patients with endometriosis, which was confirmed by laparoscopic operation, and without endometriosis (control group) via endometrial biopsy. Biopsies were performed during operative laparoscopy at Ajou University Hospital from April 2007 to June 2008. Informed consent was obtained from each participating patient, and the study was approved by the Institutional Review Board (AJIRB-GEN-SMP-10-187). Tissue specimens were acquired from patients aged 30 to 45 who were estimated to be in the mid or late proliferative phase of the menstrual cycle. Five subjects were enrolled in each group, and patients with endometriosis stage III-IV were included in the study group.

2. Reagents and antibodies

The PPAR γ agonist, rosiglitazone, was purchased from Alexin (Alexin, San Diego, CA, USA). The phosphorylated ERK (pERK) inhibitor PD98059 was purchased from Calbiochem (Calbiochem, San Diego, CA, USA). The antibodies against human pERK/ERK (mouse), hTERT (rabbit), COX-1, 2 (goat), and aromatase (goat) were purchased from Santa Cruz Biotechnology (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The antibody against b-actin was purchased from Sigma. The antibodies against goat horseradish peroxidase (HRP), mouse HRP, and rabbit HRP were purchased from Chemicon (Chemicon, Temecula, CA, USA).

3. Endometrial stromal cell isolation and primary cell culture

The protocol for cell isolation was identical to that of a previously reported method [22]. Briefly, the endometrial tissues obtained were placed in a conical tube (Falcon, Becton Dickinson, Franklin Lakes, NJ, USA) containing Dulbecco's modified Eagle's medium (DMEM)/F-12 supplemented with 10% FBS (Gibco Life Technologies, Grand Island, NY, USA) and transported to the aseptic laboratory, where the tissue was again washed several times with phosphate-buffered saline (PBS) to remove any residual blood clots and FBS. Then the tissues were minced into small pieces, incubated in 10 mL of 0.5% trypsin-EDTA (Gibco Life Technologies) for 1 hour at 37°C. After incubation, 1 mL of FBS was added to stop the enzymatic reaction. The tissues were centrifuged at 1,200 rpm for 5 minutes. The pellets were suspended with 5 mL of DMEM/F-12 and centrifuged at 1,200 rpm for 5 minutes. The pellets were resuspended in 10 mL of DMEM/F-12 containing 10% FBS, plated onto 100 mm dishes, and then allowed to adhere at 37°C for 1 hour. Non-adhering epithelial cells and blood cells were removed by rinsing the cells with PBS. The adhering ESC were cultured in DMEM/F12 containing 10% FBS, penicillin, and streptomycin. When the cells became confluent, they were dissociated with 0.5% trypsin-EDTA, harvested by centrifugation at 1,200 rpm for 5 minutes, seeded in 100 mm

dishes at 1×10^6 cells, and then incubated at 37°C in humidified 5% CO₂. After incubation for 24 hours, the media was replaced with fresh media and cultured for 72 hours under the following conditions: varying doses of rosiglitazone (PPAR γ -specific ligand, 2–40 μ M). The proliferation of ESCs and expression of aromatase were dose-dependently suppressed by rosiglitazone. We chose a 20 μ M dose of rosiglitazone for the follow-up experiments; three groups used 20 μ M of rosiglitazone, or 20 mM of the pERK inhibitor (PD98059), or 20 μ M of rosiglitazone and 20 mM of the pERK inhibitor (PD98059) together. The doses of agents added to the cultures were based on the study of Han et al. [23]. To determine the purity of the stromal cell preparations, they were characterized using immunostaining with antibodies to cytokeratin for epithelial cells (Santa Cruz Biotechnology) and vimentin for stromal cells (Santa Cruz Biotechnology). The purity of the stromal cells was over 98%, as judged by positive cellular staining for vimentin and negative cellular staining for cytokeratin.

4. Cell proliferation assays (MTT assays)

Cells were plated in 24-well plates at 5×10^4 cells/well and cultured at 37°C for 24 hours. The media was replaced with fresh media and cultured for 72 hours under the following conditions: varying doses of rosiglitazone (PPAR γ -specific ligand, 2–40 μ M), 20 μ M of rosiglitazone, and 20 mM of the pERK inhibitor (PD98059), and 20 μ M of rosiglitazone and 20 mM of the pERK inhibitor (PD98059) together. After 72 hours of treatment incubation, the cells were subjected to a cell proliferation assay by using the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) Cell Proliferation Assay Kit (R&D system, Minneapolis, MN, USA). To each well, MTT reagent (final concentration, 0.05%) was added, followed by an additional 4 hours culture. Thereafter the culture media were removed, and the formazan formed was dissolved in 150 μ L of dimethyl sulfoxide (DMSO), vortexed, and spectrophotometrically measured at 590 nm. Three sets of each group were subjected to MTT assay and each assay was repeated at least three times. The proliferation index was calculated: experimental optical density (OD) value \times 100/control OD value.

5. Western blot analysis of hTERT, aromatase, COX-2, and pERK

Western blot analysis was performed as described [24]. Briefly, cell lysates were prepared by extracting protein with lysis buffer (40 mM Tris-HCL [pH 8.0], 120 mM NaCl, 0.1% Nonidet P-40) supplemented with protease inhibitors. After sonication, the whole-cell lysate was collected by centrifugation at 14,000 rpm at 24°C for 20 minutes. The total proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose blotting membrane (Sartorius, Goettingen, Germany). The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline and incubated with primary antibodies for 1 hour at room temperature

(RT). The blots were developed by peroxidase-conjugated secondary antibody, and the proteins were visualized by an enhanced chemiluminescence procedure (Santa Cruz Biotechnology) according to the manufacturer's recommendations. Chemiluminescence measurements and semi-quantitative values were obtained by an Intelligent Dark Box II (Fujifilm, Tokyo, Japan) and Image Gauge V3.12 software (Fujifilm, Tokyo, Japan). Quantification of the western blots was performed by densitometry and normalization to β -actin and constitutive COX-1. The densitometric analyses were repeated three times using Image Gauge V3.12 (Fujifilm, Tokyo, Japan).

6. Measurement of PGE₂ concentrations in the conditioned media

To measure the production of PGE₂ during culture in various conditions for 72 hours, the cells were removed by centrifugation, and the cell-free conditioned media were collected and stored at -75°C until use. After thawing at room temperature, the medium was analyzed for immunoreactive PGE₂ by using anti-PGE₂ monoclonal antibodies (PGE₂ EIA kit, Cayman Chemicals Ann Arbor, MI, USA), according to the manufacturer's specifications, with absorbance at 450 nm in a plate reader (SpectraMax 190, Molecular Device, Sunnyvale, CA, USA). All of the experiments were repeated at least three times with different media under the same conditions to minimize intra- and interassay variations. The intra- and interassay coefficients of variation were < 3.8% and < 7.8%, respectively, for PGE₂.

7. Statistical analysis

All of the experiments were performed at least three to four times in duplicate using independent cell cultures. Where appropriate, the data are expressed as mean \pm SM and statistically analyzed with an unpaired Student's *t*-test and Kruskal-Wallis one-way analysis of variance with Duncan's test, using the computer software program SPSS ver. 11.0 for Windows (SPSS Inc., Chicago, IL, USA). *p* < 0.05 was considered statistically significant.

Results

1. ESC proliferation and hTERT expression in endometriosis

Cell proliferation was significantly increased in ESCs cultured *in vitro* from patients with endometriosis compared to those from controls (without endometriosis) by MTT assay. The protein expression of hTERT was also significantly increased in ESCs cultured *in vitro* from patients with endometriosis compared to those of the controls (Figure 1).

2. PPAR γ agonist inhibits proliferation and hTERT expression in ESCs from endometriosis

To investigate the regulation of telomerase activity and cellular pro-

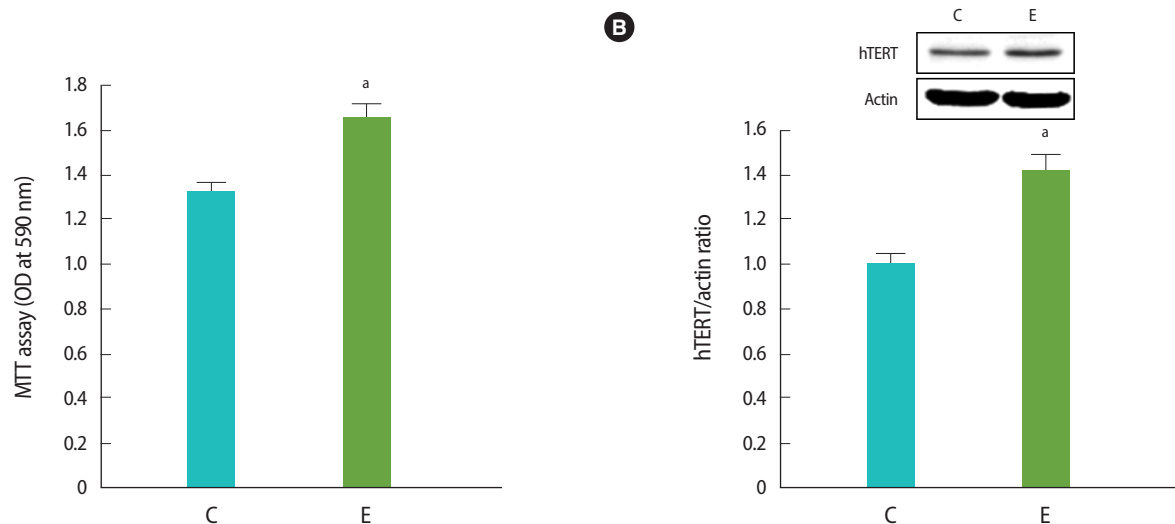


Figure 1. Endometrial stromal cell (ESC) proliferation and hTERT expression in endometriosis. (A) Cell proliferation was significantly greater in ESCs cultured *in vitro* from patients with endometriosis compared to controls (without endometriosis). ^a $p < 0.05$. (B) The hTERT protein expression was also significantly increased in ESCs cultured *in vitro* from patients with endometriosis. C, control; E, endometriosis; OD, optical density; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; hTERT, human telomerase reverse transcriptase.

liferation of eutopic endometrium by PPAR γ agonist in endometriosis, cultured ESCs from patients with endometriosis were treated with different concentrations of a PPAR γ agonist, rosiglitazone. As shown in Figure 2A, the PPAR γ agonist also dose-dependently decreased the cell proliferation. Furthermore, isolated ESCs that were obtained from patients with endometriosis resulted in a substantial induction of hTERT expression. The hTERT expression was also significantly inhibited by the PPAR γ agonist in a dose-dependent pattern (Figure 2B).

3. The effect of PPAR γ agonist on the expression of aromatase, COX-2, and PGE $_2$

To confirm the effect of PPAR γ agonist on the basic pathologic processes of endometriosis, we focused on the expression of aromatase in the estrogen biosynthetic pathway and COX-2 expression in the prostaglandin pathway. During the culture of isolated ESCs from endometriosis, stromal cells were maintained under serum-free conditions for 24 hours and then treated with PPAR γ agonist (20 μ M) for 72 hours in the rosiglitazone group. The expression of aromatase was dose-dependently inhibited after the rosiglitazone treatment (Figure 2B). COX-2 is known to be a key enzyme of PGE $_2$ production, and thus we also measured the concentration of PGE $_2$ in the conditioned cultured medium using an enzyme linked immunosorbent assay (ELISA) assay. Although the inhibition of COX-2 expression by PPAR γ agonist was not statistically significant, a subtle decrease in COX-2 could significantly suppress the PGE $_2$ formation in ESCs with endometriosis (Figure 3). We attempted to explore the effect of rosiglitazone on the MAPK signaling pathway. This pathway includes two closely related MAPKs: extracellular-signal-related kinase 1 (ERK1) and 2 (ERK2).

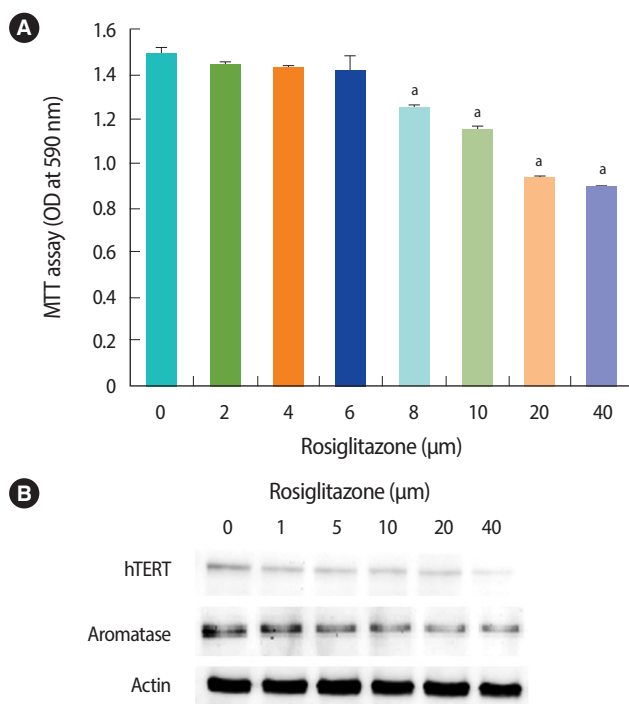


Figure 2. PPAR γ agonist inhibited telomerase activity in ESCs from endometriosis. (A) The PPAR γ agonist dose-dependently decreased cell proliferation. (B) Isolated ESCs from patients with endometriosis showed a substantial induction of hTERT expression, which was also significantly inhibited by PPAR γ agonist in a dose-dependent pattern. In addition, the expression of aromatase was dose-dependently inhibited after the PPAR γ agonist treatment. ^a $p < 0.05$. ESC, endometrial stromal cell; OD, optical density; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; hTERT, human telomerase reverse transcriptase.

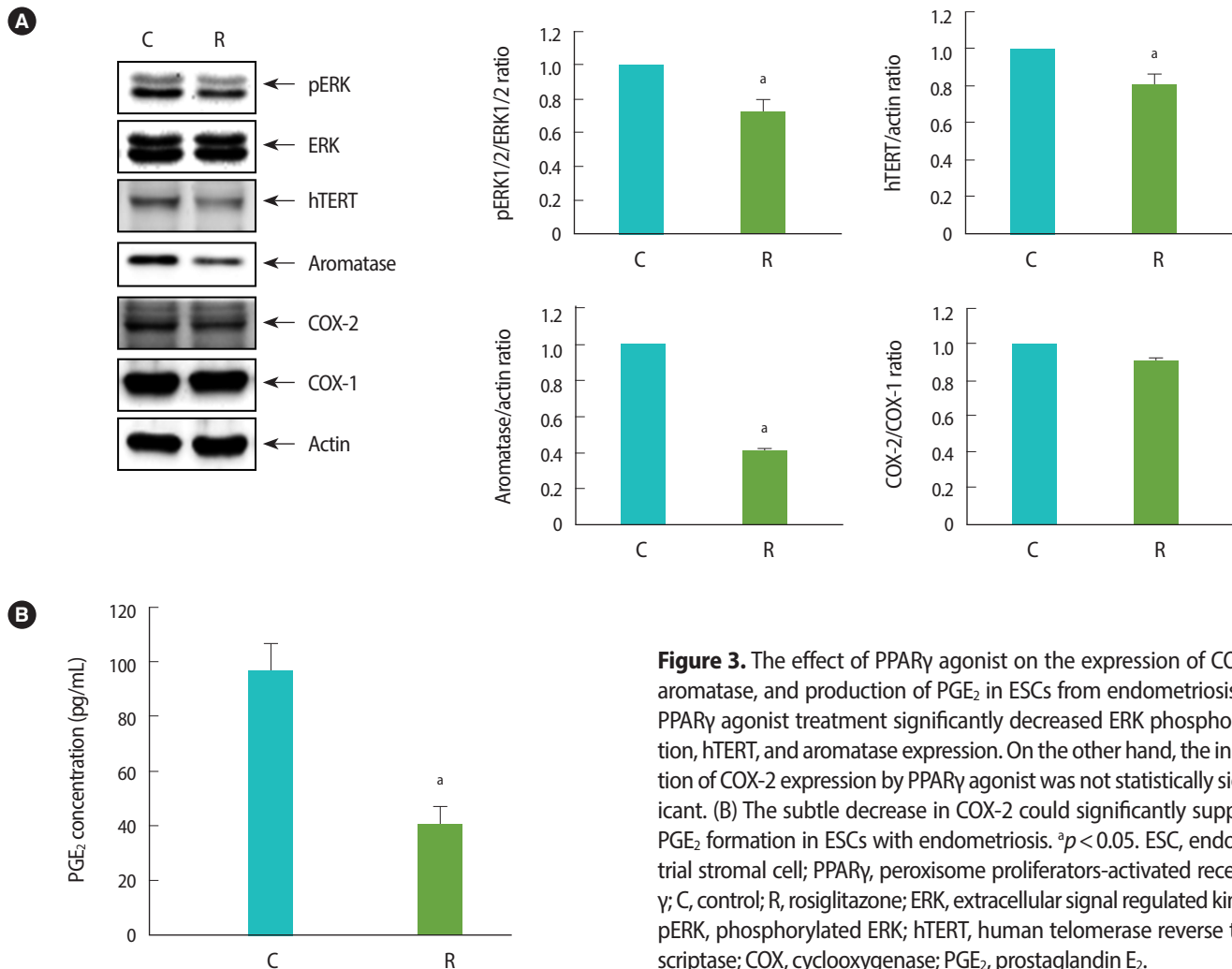


Figure 3. The effect of PPAR γ agonist on the expression of COX-2, aromatase, and production of PGE₂ in ESCs from endometriosis. (A) PPAR γ agonist treatment significantly decreased ERK phosphorylation, hTERT, and aromatase expression. On the other hand, the inhibition of COX-2 expression by PPAR γ agonist was not statistically significant. (B) The subtle decrease in COX-2 could significantly suppress PGE₂ formation in ESCs with endometriosis. ^a $p < 0.05$. ESC, endometrial stromal cell; PPAR γ , peroxisome proliferators-activated receptor γ ; C, control; R, rosiglitazone; ERK, extracellular signal regulated kinase; pERK, phosphorylated ERK; hTERT, human telomerase reverse transcriptase; COX, cyclooxygenase; PGE₂, prostaglandin E₂.

These two kinases are activated by phosphorylation; their activity was assessed by immunoblot analysis using antibodies against phosphorylated ERK1 and ERK2. As shown in Figure 3, the level of phosphorylated ERK1 and ERK2 expression was suppressed by rosiglitazone in eutopic ESCs of endometriosis.

4. Synergistic effect of PPAR γ agonist and pERK inhibitor

To further determine whether the observed inhibition of COX-2 function (PGE₂ production) in ESCs of endometriosis by PPAR γ agonist is mediated through the ERK pathway, the biologically active form of phosphorylated ERK inhibitor (PD98059) was added to PPAR γ agonist during the culture of isolated ESCs from endometriosis. Rosiglitazone treatment alone significantly inhibited cell proliferation and PGE₂ production, coupled with suppression of pERK in ESCs from endometriosis (Figure 4).

As depicted in Figure 4A, the cell proliferation of ESCs was suppressed more by rosiglitazone alone than treatment with pERK inhibitor alone. Furthermore, co-treatment of rosiglitazone and pERK inhibitor

inhibited ESC proliferation more than treatment with each agent alone. The concentration of PGE₂ in the conditioned medium was also significantly decreased by co-treatment with rosiglitazone and pERK inhibitor (PD98059) compared with rosiglitazone or pERK inhibitor alone. This means that rosiglitazone and pERK inhibitor have a synergistic effect on the suppression of cell proliferation and PGE₂ production in eutopic endometrium with endometriosis. In addition, both rosiglitazone and ERK inhibitor suppressed the expression of hTERT, aromatase, and COX-2.

Discussion

The purpose of this study was to investigate whether rosiglitazone, a PPAR γ agonist, could impede the growth of ESCs from endometriosis during *in vitro* culture, and to elucidate the effect of PPAR γ on eutopic endometrium of endometriosis. Endometriosis and ovarian cancer share common antecedent mechanisms or predisposing factors (e.g., genetic susceptibility, immune/ angiogenic dysregulation,

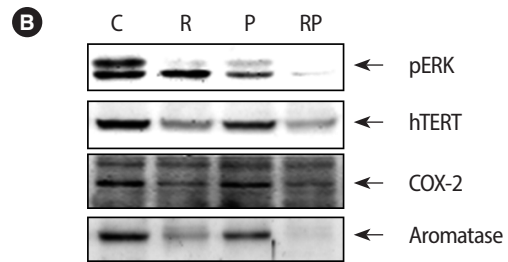
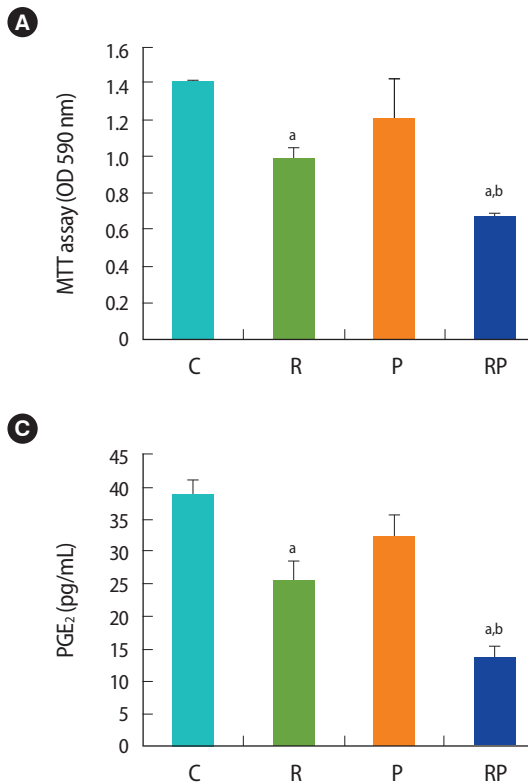


Figure 4. The synergistic effect of PPAR γ agonist and pERK inhibitor. (A) Cell proliferation was significantly inhibited by treatment with PPAR γ agonist alone, and this inhibitory effect was augmented by co-treatment with pERK inhibitor (PD98059, 20 mM). (B) Co-treatment of rosiglitazone and pERK inhibitor more suppressed the expression of hTERT, COX-2, and aromatase. (C) PPAR γ agonist significantly inhibited PGE₂ production coupled with suppression of pERK in ESCs from endometriosis (^a $p < 0.05$). C, control; R, rosiglitazone (20 μ M); P, PD98059 (20 mM); RP, rosiglitazone (20 μ M)+PD98059 (20 mM); E, endometriosis; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; OD, optical density; pERK, phosphorylated extracellular signal regulated kinase; hTERT, human telomerase reverse transcriptase; COX, cyclooxygenase; PGE₂, prostaglandin E₂; ESC, endometrial stromal cell.

environmental toxin exposure), with obvious divergence in molecular pathways downstream [7]. The eutopic endometrium of endometriosis has multiple aberrant molecular abnormalities, including the activation of oncogenic genes [25,26], overproduction of estrogen, cytokines, prostaglandins, and metalloproteinases [1], and abundant expression of angiogenic factors [27]. There have been several studies suggesting that the overexpression of fibroblast growth factors and anti-apoptotic factors such as Bcl-2 could contribute to favoring the survival and replenishment of endometriotic tissue outside of the uterus [26]. We have focused on the increased proliferation activity and previously reported an increased incidence of endometrial polyps in patients with endometriosis [28].

The replication potential and local positive feedback loop of estrogen in eutopic endometrium is essential for ESC proliferation. hTERT is a core functional component of telomerase activity. Increased telomerase expression correlates with cell proliferation [29] and most endometrial cancers [30]. It has been reported that over-expression of hTERT mRNA and telomerase activity in the endometrium of endometriosis patients and the levels of hTERT mRNA and telomerase activity increased as the disease became more severe [10]. It is suggested that the replication potential of regurgitated endometrial tissue may play an important role in the pathogenesis of endometriosis. A positive correlation has been shown between hTERT mRNA expression by quantitative real-time RT-PCR and telomerase activity by TRAP assay in endometrial tissue [10,11]. Therefore, we investigated the

hTERT protein expression of cultured ESCs as an alternative to observing the telomerase activity in woman with endometriosis.

PGE₂ was found to be the most potent known inducer of aromatase activity in ESCs [5], and estrogen increased PGE₂ formation by stimulating COX-2 enzyme in ESCs *in vitro* [31]. Thus, a positive feedback loop for the continuous local production of estrogen and PGE₂ has been established, leading to the proliferative and inflammatory activity of endometriosis. In the eutopic endometrium of women with endometriosis, there is a subtle increase in COX-2 activity and detectable aromatase activity. Therefore, moderate PGE₂ and low estradiol can be detected locally. In ectopic endometriotic tissue, full-blown molecular abnormalities include high COX-2 and aromatase levels. Excessive formation of PGE₂ in endometrial and endometriotic tissues can cause severe menstrual cramps and chronic pelvic pain. The estradiol level in endometrial tissue is high because estradiol is overproduced by aromatase and is not metabolized owing to deficient HSD17B2 activity [32]. Yoshino et al. [33] reported that ERK is present in endometriotic tissues and is activated by pro-inflammatory agents. They also showed that IL-1-induced COX-2 expression was suppressed by ERK inhibitors. This finding suggests that the ERK pathway may play a possible pathophysiologic role in endometriosis, acting as an intracellular signal transducer.

PPAR γ agonists have been shown to mediate the pro-apoptotic activity of vascular smooth muscle cells [34], and inhibit telomerase activity during the proliferative response after vascular injury [16], and

have thus provided a new therapeutic approach to preventing restenosis. Therefore, we attempted to elucidate the effect of PPAR γ agonist on hTERT expression, and the mechanisms underlying the interaction of PPAR γ agonist and production of the local cytokine and PGE $_2$ pathway through ERK phosphorylation in the eutopic endometrium of endometriosis. We observed that PPAR γ agonist dose-dependently decreased the cell proliferation and hTERT expression. The expression of aromatase was also dose-dependently inhibited after rosiglitazone treatment. Our results agree with the finding that PPAR γ agonist decreases aromatase activity in cultured human granulosa cells [35]. In addition, PPAR γ agonist decreased phosphorylated ERK in the eutopic endometrium of endometriosis. The release of PGE $_2$ by cultured ESCs was significantly decreased. The expression of aromatase and COX-2 was significantly inhibited after the addition of rosiglitazone, and this resulted in a considerable decrease in PGE $_2$ production in the culture medium. These changes were decreased more by adding ERK inhibitor. This means that rosiglitazone and pERK inhibitor have a synergistic effect on the suppression of cell proliferation and PGE $_2$ production in eutopic endometrium with endometriosis. Similarly, we have also noted that PPAR γ agonist is linked to a negative role in ERK signaling, which is activated by TGF- β in the decidualization process of cultured normal human ESCs [21].

In fact, few animal studies concerning PPAR γ agonist were able to find a regression of endometriotic lesions. It has been shown that a PPAR γ agonist, rosiglitazone, with the ability to modulate inflammatory cells and cytokine formation, affected the induction of endometriosis negatively in an experimental rat model [19], and the regression of experimental endometriosis in rat [20,36] and baboon models [37]. PPAR γ ligands reduced monocyte migration in an *in vitro* study of the peritoneal fluid of women with endometriosis [38]. Ciglitazone has been shown to decrease the number of peritoneal macrophages in a mouse model [39] and to decrease attachment of endometrial cells to peritoneal mesothelial cells in an *in vitro* model of early endometriotic lesions [40]. In our study, we found that PPAR γ agonist could diminish PGE $_2$ production from eutopic endometrium, which was thought to be the cellular origin of endometriosis. This could be explained by a regression of the endometriotic lesion in an animal model by a PPAR γ agonist, which resulted in the decrease in hTERT, aromatase, and COX-2 expression. In addition, several studies have shown that PPAR γ agonists could decrease aromatase activity in cultured human granulosa cells [35] and immunomodulate endometrial epithelial and stromal cells [38,41]. PPAR γ agonists in animal models of endometriosis have shown promise; further studies could be undertaken in efforts to determine the clinical efficacy of PPAR γ agonists as a potential treatment option for endometriosis.

The safety of PPAR γ agonists in early pregnancy has been suggested by recent studies. Studies in mice have shown that rosiglitazone

does not have an adverse impact on embryo or neonatal development [42], and may even have beneficial effects on blastocyst quality in female mice with diet-induced obesity [43]. A small case series of pregnant women with polycystic ovary syndrome who took rosiglitazone during the first trimester of pregnancy demonstrated no evidence of developmental or congenital abnormalities [44]. It should be mentioned that the meta-analysis suggesting that the PPAR γ agonist rosiglitazone increases the risk of cardiac ischemia among type 2 diabetics [45] shifted the potential extension of the results of our study to the clinical use of pioglitazone, a PPAR γ agonist that has been shown to have a favorable safety profile in type 2 diabetics, in terms of cardiovascular health [46].

Overall, our study suggests that the PPAR γ agonist rosiglitazone suppressed cell proliferation and hTERT expression in *in vitro* cultures of eutopic ESCs of endometriosis. Moreover, the expression of aromatase and COX-2 was significantly inhibited after the rosiglitazone treatment, resulting in a considerable decrease in PGE $_2$ production in culture medium through the partial inhibition of the ERK pathway. Further clinical studies in humans could be conducted on whether these results could be translated into reduced pelvic pain or endometriotic lesion size, such as endometrioma, with rosiglitazone treatment.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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