TGF-β1 induces proteinase-activated receptor 2 (PAR2) expression in endometriotic stromal cells and stimulates PAR2 activation-induced secretion of IL-6

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BACKGROUND: Proteinase-activated receptor 2 (PAR2) is a G-protein-coupled receptor that is activated by several serine proteases. PAR2 activation in endometriotic stromal cells (ESCs) has been implicated in the development of endometriosis but the regulatory mechanism of PAR2 expression in ESC is unknown. Our objective was to study the mechanism by which PAR2 expression may be regulated in endometriotic lesions.

METHODS: Primary cultures of ESCs were treated with transforming growth factor-β (TGF-β) 1, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and the expression of PAR2 was examined by real-time quantitative PCR. ESCs pretreated with or without TGF-β1 were treated with PAR2 agonist peptide (PAR2AP) and the secretion of the pro-endometriotic cytokine, IL-6, was measured using a specific enzyme-linked immunosorbent assay. Effects of TGF-β type 1 inhibitor, SB431542, and PAR2 small interfering RNA (siRNA) on the TGF-β1 stimulation of PAR2 gene expression and PAR2AP-induced IL-6 secretion were also evaluated. To study intracellular signaling, effects of inhibitors of mitogen-activated protein kinases (MAPKs) and phosphoinositide 3-kinase (PI3K) and of Smad4 siRNA on the TGF-β1-induced PAR2 gene expression were studied.

RESULTS: Only TGF-β1, but neither TNF-α nor IL-1β, increased gene expression of PAR2. Activation of PAR2 with PAR2AP increased the secretion of IL-6 from ESCs. As expected, TGF-β1 pretreatment dose-dependently enhanced the PAR2AP-induced increase in IL-6 secretion from ESCs. Treatment of ESCs with the TGF-β type 1 inhibitor, SB431542, inhibited both TGF-β1-stimulation of PAR2 gene expression and PAR2AP-induced IL-6 secretion. Transfection of ESCs with PAR2 siRNA produced a similar inhibition of IL-6 secretion. The TGF-β1-induced increase in PAR2 gene expression was repressed by inhibition of p38 MAPK, p42/44 MAPK or PI3K, but not by knockdown of Smad4 expression.

CONCLUSIONS: In view of significant roles of PAR2 and IL-6 in endometriosis, the TGF-β1-induced increase in PAR2 expression may be an elaborate mechanism that augments the progression of the disease.

Key words: endometriosis / TGF-β1 / proteinase-activated receptor / interleukin-6

Introduction

Endometriosis is defined by the presence of viable endometriotic tissue outside the uterus and remains an incompletely understood disease. Endometriosis adversely affects the health of women of reproductive age, causing pain and infertility (Momoeda et al., 2002; Osuga et al., 2002). Although numerous studies have been conducted on the pathophysiology of the disease, its mechanism of progression is poorly understood. Multiple lines of evidence indicate that endometriosis is a chronic inflammatory disease and both immune and inflammatory responses contribute to the development of the disease. The immune and inflammatory responses are induced by interactions of
endometriosis-associated immune cells with endometriotic cells through various inflammatory substances, such as cytokines, chemokines, proteases, prostaglandins and growth factors. A number of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), interleukin (IL)-1, IL-6, IL-8 and IL-17, are suggested to play important roles, such as cell proliferation and angiogenesis, in promoting the disease (Lebovic et al., 2001; Osuga, 2008; Osuga et al., 2008, 2011).

Proteinase-activated receptor 2 (PAR2) is a G-protein-coupled receptor that is activated by cleavage within its extracellular N-terminal domain (Macfarlane et al., 2001). We have previously reported that PAR2 activation stimulates the secretion of IL-6 and IL-8 in endometriotic stromal cells (ESCs) (Hirota et al., 2005a). This response suggests that PAR2 has a functional role in endometriosis-associated inflammation. Moreover, PAR2 activation induces proliferation of ESCs, indicating that PAR2 activation may directly relate to the growth of the endometriotic lesion (Hirota et al., 2005a). In a mouse model of endometriosis, both the number and the total weight of endometriotic lesions were significantly decreased in the PAR2-deficient mice compared with the wild-type mice. Interestingly, concentrations of IL-6 and monocyte chemotactic protein-1 were decreased in the peritoneal fluid and the serum of the PAR2-deficient mice, suggesting alleviated inflammation in the peritoneal cavity of the mice (Osuga et al., 2008). These findings underscore the possible pivotal role of PAR2 in endometriosis. In the eutopic endometrium, PAR2 expression is increased during the menstrual phase (Hirota et al., 2005b), which might contribute to the implantation of endometrial fragments in the retrograde menstruation to the peritoneum. PAR2 activation is induced by proteases from neutrophils and mast cells, which are both observed in endometriotic tissues. PAR2 is also activated by the coagulation protein-1 were decreased in the peritoneal fluid and the serum of the PAR2-deficient mice, suggesting alleviated inflammation in the peritoneal cavity of the mice (Osuga et al., 2008). These findings underscore the possible pivotal role of PAR2 in endometriosis. Despite the observed effects of PAR2 in endometriosis, the regulation of PAR2 expression in endometriotic tissues remains unknown. TNF-α and IL-1β both increase PAR2 expression in neurons (Noorbakhsh et al., 2005), whereas TGF-β increases PAR2 expression in fibroblasts (Materazzi et al., 2007). Interestingly, TNF-α, IL-1β and TGF-β are all implicated in the development of endometriosis. In the present study, we found that TGF-β1, but neither IL-1β nor TNF-α, increased the gene expression of PAR2 in ESCs. This finding prompted us to investigate further the TGF-β1-induced expression of PAR2 in endometriosis. Therefore, we examined the effect of TGF-β1 on PAR2 activation-induced IL-6 secretion in ESCs. IL-6 is a representative pleiotropic cytokine involved in the development of endometriosis (Witz, 2000; Salmassi et al., 2008). We also studied the possible intracellular mechanism by which TGF-β1 increases PAR2 expression in ESCs.

**Materials and Methods**

**Patients and samples**

Endometriotic tissues were obtained from patients with ovarian endometriomas undergoing laparoscopy. The diagnosis of endometriosis was confirmed by histopathological examination. Laparoscopic excision of ovarian endometriomas was performed as follows. After inspection of the pelvis, the ovary was freed from any adhesions. The endometrioma cyst wall was stripped away from the normal ovarian tissue gently and completely. Endometriotic tissue samples obtained from the excised endometrioma cyst wall were transported to the laboratory in DMEM/Ham’s F12 medium (DMEM/F12; Invitrogen, Rockville, MD, USA) on ice under sterile conditions. All of the women had regular menstrual cycles, and none had received hormonal treatment for at least 6 months before surgery. This experimental procedure was approved by the Institutional Review Board of the University of Tokyo and signed informed consent for the use of the endometriotic tissues was obtained from each woman.

**Isolation and culture of human ESCs**

Isolation and culture of human ESCs were conducted as described previously (Hirota et al., 2005a,c; Hirata et al., 2008). Briefly, fresh endometriotic tissues collected in sterile medium were rinsed to remove blood cells and then were minced into small pieces and incubated in DMEM/F-12 containing type I collagenase (0.25%; Sigma, St Louis, MO, USA) and deoxyribonuclease I (15 U/ml; Takara, Tokyo, Japan) for 120 min at 37°C. The resulting dispersed endometriotic cells were separated by filtration through a 100 and 70 μm nylon cell strainers (BD, Franklin Lakes, NJ, USA). ESCs in the filtrate were collected by centrifugation and resuspended in phenol-red free DMEM/F-12 containing 5% charcoal-stripped fetal bovine serum (FBS), 100 U/ml penicillin, 0.1 mg/ml streptomycin and 0.25 μg/ml amphotericin B. ESCs were seeded in a 100 mm culture plate and kept at 37°C in a humidified 5% CO₂–95% air atmosphere. At the first passage, the cells were plated into 12- or 48-well culture plates (BD) at a density of 2 × 10³ cells/ml. The purity of the ESC population was more than 95%, as judged by positive cellular staining for vimentin and negative cellular staining for cytokeratin, CD45 and von Willebrand factor.

**Treatment of ESCs**

When the ESC culture reached 70–80% confluence in 1 or 2 days, media were removed and replaced with fresh media containing 2% charcoal-stripped FBS and antibiotics. After culturing for an additional 12 h, the cells were ready for use in the experiments. To examine the effect of cytokines on PAR2 gene expression, ESCs were incubated with TGF-β1 (10 ng/ml), IL-1β (10 ng/ml) and TNF-α (10 ng/ml) (all cytokines were from R&D Systems, Minneapolis, MN, USA) for 6 h. We used PAR2 agonist peptide (PAR2AP; SLIGKV, BACHEM, Bubendorf, Switzerland) for the activation of PAR2 (Hirota et al., 2005a). PAR2AP comprised the unmasked amino-terminal peptide of PAR2 cleaved by the activating protease. To examine the effect of TGF-β1 on IL-6 secretion induced by PAR2AP, ESCs were pretreated with TGF-β1 (10 ng/ml) for 24 h and then incubated with PAR2AP (30 μM) for 24 h. To examine the effect of inhibition of type 1 TGF-β receptor on gene expression of PAR2, ESCs were incubated with or without SB431542 (10 μM) (Calbiochem, La Jolla, CA, USA), and with TGF-β1 (10 μg/ml) for 6 h. To examine the effect of SB431452 on PAR2AP-induced IL-6 secretion, ESCs were pretreated with or without SB431452 (10 μM) and with TGF-β1 (10 ng/ml) for 24 h and then incubated with PAR2AP (30 μM) for 24 h. To examine the effect of inhibition of type 1 TGF-β receptor on gene expression of PAR2, ESCs were incubated with or without SB431452 (10 μM) and with TGF-β1 (10 ng/ml) for 24 h and then incubated with PAR2AP (30 μM) for 24 h. To examine the effect of inhibitors of mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) on TGF-β1-induced gene expression of PAR2, ESCs were pretreated with SB202190 (10 μM), PD98059 (25 μM), SP600125 (10 μM) or LY294002 (20 μM) (inhibitors of p38 MAPK, p42/44 MAPK, stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) and PI3K, respectively; Calbiochem) for 30 min before treatment with TGF-β for 6 h.
Small interfering RNA
The small interfering RNA (siRNA) constructs used were obtained as ON-TARGET plus SMART pool PAR2 (L-005095-00-0005) and Smad4 (L-003902-00-0005) from Dharmacon (Lafayette, CO, USA). The non-targeting siRNA control, ON-TARGET plus siCONTROL non-targeting pool (D-001810-10-05), was also obtained from Dharmacon. Cells were transfected with 30 nmol/l siRNA for 24 h in Opti-MEM I using Lipofectamine RNAi max according to the manufacturer’s protocol. After transfection, the medium was removed and replaced with fresh medium containing 5% charcoal-stripped FBS and antibiotics for 24 h. The cells were then treated with TGF-β1 and PAR2AP as described above.

RNA extraction, RT and real-time quantitative PCR
RNA extraction, RT and real-time quantitative PCR were performed as described previously (Takemura et al., 2007; Hirata et al., 2008). Total RNA was extracted from cultured ESCs using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Real-time quantitative PCR and data analysis were performed using a LightCycler (Roche Diagnostic GmbH, Mannheim, Germany), according to the manufacturer’s instructions. Expression of PAR2 and Smad4 mRNA was normalized to RNA loading for each sample using human glyceraldehyde-3-phosphatedehydrogenase (GAPDH) mRNA as an internal standard. The PAR2 primers chosen (sense, 5′-CTGCATCTGCTCTCAGTGGA-3′; antisense, 5′-ACAGAGAGGAAGTCAGCAGC-3′) amplified a 181 bp fragment. The Smad4 primers chosen (sense, 5′-TGCTCTGCGAAAGGATTT-3′; antisense, 5′-ACTGCCAGGCTCGACTTG-3′) amplified a 431 bp fragment. The PCR conditions were as follow: for PAR2, 40 cycles at 95°C for 10 s, 64°C for 10 s and 72°C for 10 s; for Smad4, 40 cycles at 95°C for 10 s, 64°C for 10 s and 72°C for 18 s; for GAPDH, 30 cycles at 95°C for 10 s, 64°C for 10 s and 72°C for 18 s. All of the PCR experiments were followed by melting curve analysis.

Measurement of IL-6 protein
The concentration of IL-6 in the conditioned media was measured using a specific ELISA kit (R&D Systems). The sensitivity of the assay was 3.12 pg/ml, and the intra- and inter-assay coefficients of variation were less than 5%.

Statistical analysis
Data were analyzed by ANOVA, followed by post hoc analysis for multiple comparisons, or Student’s t-test, appropriately. A value of P < 0.05 was considered significant.

Results
Effects of TGF-β1, TNF-α and IL-1β on gene expression of PAR2
TGF-β1 increased gene expression of PAR2, whereas neither TNF-α nor IL-1β affected PAR2 expression (Fig. 1A). The TGF-β1-induced PAR2 expression was dose-dependent between 1 and 10 ng/ml, the increase being significant from 1 ng/ml (Fig. 1B).

Effects of TGF-β1 on PAR2AP-induced IL-6 secretion
Although PAR2AP alone increased IL-6 secretion by 2.8-fold, TGF-β1 pretreatment dose-dependently enhanced PAR2AP-induced IL-6 secretion, with a total increase of 9.8-fold observed at 10 ng/ml TGF-β1 (Fig. 2).

Effects of SB431542 and PAR2 siRNA on TGF-β1-induced gene expression of PAR2 and on TGF-β1 stimulation of PAR2AP-induced IL-6 secretion
The TGF-β1 type I inhibitor, SB431542, suppressed the TGF-β1-induced expression of PAR2 (Fig. 3A). SB431542 also suppressed the TGF-β1 stimulation of PAR2AP-induced IL-6 secretion (Fig. 3B). Knockdown of PAR2 expression using PAR2 siRNA remarkably reduced PAR2 mRNA levels (Fig. 4A). Similar to the treatment with SB431542, PAR2 siRNA treatment inhibited the TGF-β1 stimulation of PAR2AP-induced IL-6 secretion (Fig. 4B).

Effects of MAP kinase inhibitors, PI3K inhibitor and Smad4 siRNA on TGF-β1-induced gene expression of PAR2
The Smad pathway, several MAPK pathways and PI3K pathway are typical intracellular signaling pathways activated by TGF-β1. To examine whether these pathways are involved in TGF-β1-induced gene expression of PAR2, ESCs were treated with MAPK inhibitors and Smad4 siRNA. The p38 MAPK, p42/44 MAPK and PI3K inhibitors (SB202190, PD98059 and LY294002 respectively) significantly

Figure 1 TGF-β1-induced increase in PAR2 mRNA expression in ESCs. (A) ESCs were cultured with TGF-β1 (10 ng/ml), TNF-α (10 ng/ml) and IL-1β (10 ng/ml) for 6 h. (B) ESCs were cultured with different concentrations of TGF-β1 for 6 h. Total RNA isolated from ESCs was reverse transcribed and amplified by real-time PCR using primers for PAR2. Values were calculated by subtracting data for signal threshold cycles (C) of the internal standard (GAPDH) from C values for PAR2. Data are the mean ± SEM of six (A) and five (B) independent experiments using different ESCs. The data were analyzed by ANOVA, followed by post hoc analysis for multiple comparisons. *P < 0.05 versus control.
TGF-β enhances PAR2-mediated IL-6 secretion

Figure 2 TGF-β1-induced increase in PAR2AP-induced ESC secretion of IL-6. ESCs were pretreated with TGF-β1 (0, 1, 5 and 10 ng/ml) for 24 h and subsequently incubated with or without 30 µM PAR2AP for 24 h. At the end of the incubation period, the conditioned medium was collected and assayed for IL-6 by ELISA. The values are presented as the mean ± SEM of four separate cultures. *p < 0.05 versus ESCs stimulated with PAR2AP but without TGF-β1 pretreatment. The result is representative of three repeated experiments using samples from three different women.

Figure 3 Effects of SB431542 on the TGF-β1-induced increase in PAR2 mRNA expression and in PAR2AP-induced IL-6 secretion from ESCs. (A) ESCs were cultured with or without SB431542 (10 µM) and TGF-β1 (10 ng/ml) for 24 h. Total RNA isolated from ESCs was reverse transcribed and amplified by real-time PCR using primers for PAR2. Values were calculated by subtracting data for signal threshold cycles (C_t) of the internal standard (GAPDH) from C_t values for PAR2. The values are presented as the mean ± SEM of three independent experiments. The data were analyzed by Student’s t-test. (B) ESCs were pretreated with or without SB431542 (10 µM) and TGF-β1 (10 ng/ml) for 24 h and subsequently incubated with PAR2AP (30 µM) for 24 h. At the end of the incubation period, the conditioned media were collected and assayed for IL-6 by ELISA. The values are presented as the mean ± SEM of four separate cultures. Data are shown as the fold change in IL-6 concentrations in ESCs not pretreated with SB431542 and TGF-β1 and not stimulated with PAR2AP. The data were analyzed by ANOVA, followed by post hoc analysis for multiple comparisons. The result is representative of three repeated experiments using samples from three different women. (A and B) Different letters denote significant differences between groups (p < 0.05).

Figure 4 Effects of PAR2 siRNA on TGF-β1-induced increase in PAR2 mRNA expression and in PAR2AP-induced ESC secretion of IL-6. (A) ESCs were transfected with 30 nmol/l PAR2 siRNA or negative control siRNA for 24 h. After transfection, the medium was removed and replaced with medium containing 5% charcoal-stripped FBS and antibiotics for 24 h. Thereafter, ESCs were cultured with or without TGF-β1 (10 ng/ml) for 24 h. Total RNA isolated from ESCs was reverse transcribed and amplified by real-time PCR using primers for PAR2. Values were calculated by subtracting data for signal threshold cycles (C_t) of the internal standard (GAPDH) from C_t values for PAR2. The values are presented as the mean ± SEM of three independent experiments using different ESCs. The data were analyzed by Student’s t-test. (B) ESCs were transfected with 30 nmol/l PAR2 siRNA or negative control siRNA for 24 h. After transfection, the medium was removed and replaced with the medium containing 5% charcoal-stripped FBS and antibiotics for 24 h. Thereafter, ESCs were transfected with or without TGF-β1 (10 ng/ml) for 24 h. At the end of the incubation period, the conditioned medium was collected and assayed for IL-6 by ELISA. The values are presented as the mean ± SEM of four separate cultures. Data are shown as fold changes in IL-6 concentrations in ESCs pretreated with control siRNA but without TGF-β1, and not stimulated with PAR2AP. The data were analyzed by ANOVA, followed by post hoc analysis for multiple comparisons. The result is representative of three repeated experiments using samples from three different women. (A and B) Different letters denote significant differences between groups (p < 0.05).

Discussion

In the present study, we demonstrated that TGF-β1, but neither TNF-α nor IL-1β, increased gene expression of PAR2. TGF-β1 dose-dependently increased the secretion of IL-6 in PAR2AP-stimulated ESCs. SB431542, an inhibitor of the TGF-β receptor, inhibited the TGF-β1-induced increase in gene expression of PAR2 in ESCs and the TGF-β1-augmented IL-6 secretion from PAR2AP-stimulated ESCs. Likewise, PAR2 siRNA inhibited the TGF-β1-induced increase in gene expression of PAR2 in ESCs and the TGF-β1-augmented diminishing TGF-β1-induced PAR2 gene expression (Fig. 5A). In contrast, neither the SAPK/JNK inhibitor (SP600125) nor treatment with Smad4 siRNA had any effect on TGF-β1-induced PAR2 gene expression, although Smad4 siRNA markedly decreased gene expression of Smad4 (Fig. 5B).
TGF-β may play multiple roles in different stages of the progression of endometriosis (Omwandho et al., 2010). The present study demonstrates a new role of TGF-β1 in the development of endometriosis, in the induction of PAR2 expression in ESCs. The increase in PAR2 expression consequently enhanced IL-6 secretion from PAR2AP-stimulated ESCs. Given that the proteinases and the coagulation product that activate PAR2 are present in endometriotic tissues, this sequence may partly explain the increased expression of IL-6 in both endometriotic tissue and in peritoneal fluid of women with endometriosis (Salmassii et al., 2008; Velasco et al., 2010). The elevation in TGF-β levels in endometriotic tissues is therefore likely to contribute to this sequence of events (Tamura et al., 1999; Komiyama et al., 2007).

IL-6 is a multifunctional cytokine that is involved in numerous immunological and proliferative responses in endometriosis (Witz, 2000). In particular, IL-6 increases aromatase activity, haptoglobin production and hepatocyte growth factor production in endometriotic cells and/or endometriotic cells (Piwa et al., 2001; Khan et al., 2005; Velasco et al., 2006; Sharpe-Timms et al., 2010). IL-6 is also known to stimulate the proliferation of ESCs (Khan et al., 2005). These findings indicate that IL-6 stimulates the progression of endometriosis via various events such as cell proliferation, angiogenesis and immunomodulation. Therefore, the TGF-β1-stimulated increase in PAR2 expression and the resulting increase in IL-6 is a possible mechanism by which TGF-β1 can amplify PAR2-mediated disease progression. This hypothesis is also consistent with the previous finding that PAR2 activation stimulates the proliferation of ESCs (Hirotta et al., 2005a,b,c). In addition, increased IL-6 production via a TGF-β1-stimulated increase in PAR2 expression might contribute to endometriosis-associated infertility, because IL-6 is suggested to be a causative factor for infertility in endometriosis (Gomez-Torres et al., 2002; Yoshida et al., 2004; Deura et al., 2005).

The effect of TGF-β1 on PAR2 expression might provide a novel insight in the pathogenesis of endometriosis. Immune cells are an important component of endometriotic tissues and are involved in the development of the disease. In particular, our recent studies suggest that Th2 cells and Th17 cells contribute to disease progression by inducing inflammation and cell proliferation (Hirotta et al., 2008; OuYang et al., 2008, 2010). The differentiation of Th cells is under the strict control of cytokines, with Th17 cells being induced from naïve Th cells by TGF-β1 in combination with IL-6. Without IL-6, naïve Th cells will differentiate into regulatory T cells under the influence of TGF-β1 (Miossec et al., 2009). Therefore, we hypothesize that TGF-β1 stimulates the environment to aid the development of Th17 cell by increasing PAR2 activation-induced secretion of IL-6 in endometriotic tissues. This process could be potentiated by enhancement of PAR2 activation by the proteolytic enzymes produced by neutrophils activated by Th17 cells (Miossec et al., 2009). In this way, TGF-β1 may co-operate with Th17 cells to stimulate disease progression. Further studies are warranted to corroborate the notion.

TGF-β utilizes multiple signaling pathways to stimulate different cells. Smad4 is essential for TGF-β signal transduction (Prud’homme, 2007), but suppression of Smad4 expression by siRNA did not inhibit the TGF-β1-induced increase in PAR2 expression in ESCs. In contrast, a p38 MAPK inhibitor and a p42/44 MAPK inhibitor suppressed the effect of TGF-β1. The present findings indicate that the activation of IL-6 secretion from PAR2AP-stimulated ESCs. SB202190, a p38 MAPK inhibitor, PD98059, a p42/44 MAPK inhibitor, and LY294002, a PI3K inhibitor, suppressed the TGF-β1-induced expression of PAR2. Suppression of Smad4 expression by the siRNA had no effect on TGF-β1-induced gene expression of PAR2.

It is interesting that neither IL-1β nor TNF-α increased gene expression of PAR2 in ESCs, compared with the stimulation by both molecules of PAR2 expression in neurons, osteoarthritis chondrocytes and osteoblasts (Noorbakhsh et al., 2005; Xiang et al., 2006; Boileau et al., 2007; Amiable et al., 2009). We observed that TGF-β1 increased PAR2 expression in ESCs, which is consistent with TGF-β1 stimulation of PAR2 expression in human dermal fibroblasts (Materazzi et al., 2007). Therefore, PAR2 expression appears to be differentially regulated in different cell types.
TGF-β1 expression.

(Tamura et al., 2004). Presumably, the activation of p38 MAPK and p42/44 MAPK in response to TGF-β1, but neither TGF-β2 nor TGF-β3, on PAR2 expression in ESCs. As TGF-β2 and TGF-β3 have been reported to be increased in endometriotic tissues (Tamura et al., 1999), it would be interesting also to study the effect of these molecules.

In summary, the present study demonstrated that TGF-β1 enhanced PAR2 expression and, as a consequence, increased PAR2-activation-induced IL-6 secretion from ESCs. In light of the multiple roles of PAR2 in promoting the development of endometriosis, TGF-β1 may accelerate disease progression by up-regulating PAR2 expression.

Authors’ roles


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