Reproductive Sciences
2017, Vol. 24(1) 124-132

DOI: 10.1177/1933719116650751
journals.sagepub.com/home/rsx
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PRL-3 Is Involved in Estrogen- and IL-6–Induced Migration of Endometrial Stromal Cells From Ectopic Endometrium

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Abstract

Objective: To investigate the role of phosphatase of regenerating liver-3 (PRL-3) in the 17β-estradiol (E2)- and interleukin 6 (IL-6)-induced migration of endometrial stromal cells (ESCs) from ectopic endometrium. **Methods:** Ectopic endometrial tissues were collected from patients with endometriosis, and PRL-3 expression in ectopic and eutopic endometrium was examined by immunohistochemistry. Endometrial stromal cells isolated from ectopic endometrium were treated with E2, progesterone (P), IL-6, or sodium orthovanadate (Sov) to inhibit PRL-3. Total RNA and protein were extracted from ESCs after treatment for quantitative real-time polymerase chain reaction and Western blot analyses. Cell migration was assessed using a scratch wound assay. **Results:** Phosphatase of regenerating liver 3 protein was highly expressed in the endometrial glandular cells (EGCs) and ESCs in ectopic endometrium, whereas its weak expression was observed only in EGCs in eutopic endometrium. Both E2 and IL-6 treatment significantly increased PRL-3 messenger RNA and protein expression, and P treatment significantly inhibited PRL-3 expression. However, E2-induced PRL-3 expression in ESCs from ectopic endometrium was significantly blocked by IL-6 antibody. Moreover, E2- and IL-6–enhanced cell migration was completely abrogated by Sov treatment. Furthermore, Sov treatment could significantly promote PTEN expression but inhibit E2- and IL-6–induced p-AKT activation. **Conclusion:** Phosphatase of regenerating liver 3 plays a key role in the E2- and IL-6–induced migration of ESCs from ectopic endometrium, a process that is involved in the PTEN-AKT signaling pathway.

Keywords

E2, IL-6, PRL-3, AKT signaling, endometriosis, cell migration

Introduction

Endometriosis is a common gynecologic disorder that is classically defined as the presence of endometrial glands and stromal in ectopic locations, such as the pelvic peritoneum, ovaries, and retrocervical septum.[1,2] Endometriosis affects 6% to 10% of women at reproductive age and can cause chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility.[3] The prevalence of endometriosis is higher in infertile women (20%-50%) and in women and adolescents with chronic pelvic pain and dysmenorrhea (25%-70%).[4] Although the underlying pathophysiology of endometriosis remains elusive, it has been demonstrated that endometriosis is associated with genetic factors, hormonal influences, specifically estrogenic stimulation, and environmental insults, such as exposure to endocrine disruptors.[5] Endometriosis possesses mixed traits of benign disease and malignancy. The pathogenesis of endometriosis is related to uncontrolled cell proliferation and local and distant migration and metastasis.[6,7] Thus, the increased migratory capacity of endometrial stromal cells (ESCs) is the first prerequisite for the development of endometriosis, although the precise molecular mechanisms are still largely unknown.

Endometriosis is also an estrogen-dependent gynecological disease. Aberrant expression and regulation of estrogen receptor (ER) and progesterone receptor (PR) are observed in the different types of endometriosis.[8] Compared to normal uterine tissues, the expression of ERα messenger RNA (mRNA) and protein is decreased in endometriosis lesions, whereas the expression levels of ERβ mRNA and protein in ovarian endometriosis were found to be similar.[9,10] The expression of PRA

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and PRB mRNA and protein is decreased or absent in ovarian endometriosis, and the expression of PRA/B mRNA and protein fails to show a consistent and regulated pattern in peritoneal endometriosis.[11,12] Interestingly, Zeitoun et al demonstrated that aberrant P450arom expression in endometriotic tissue in contrast to eutopic endometrium is involved in the local biosynthesis of estrogen that causes the growth of these lesions.[13] It has been demonstrated that rapid activation of the ERK1/2 and PI3K/AKT intracellular signaling cascades by growth factors and estrogens is involved in the migration of normal ESCs.[14] Moreover, endometriosis is a pelvic inflammatory process that affects the secretion of various local products such as growth factors and cytokines in the peritoneal environment.[15] Elevated levels of cytokines and other factors, including C-reactive protein, serum amyloid A, tumor necrosis factor (TNF) α, monocyte chemoattractant protein (MCP) 1, interleukin (IL)-6, IL-8, and chemokine (C–C motif) receptor 1, are found in the peritoneal fluid and peripheral blood of patients with endometriosis.[16] Khan et al observed significantly increased secretion of IL-6 and TNF-α in peritoneal macrophages in response to 17β-estradiol (E2).[17] However, the precise molecular mechanisms underlying the roles of estrogen and progesterone (P) in the pathogenesis of endometriosis are still not well elucidated.

Phosphatase of regenerating liver 3 (PRL-3) is a member of the phosphatases of regenerating liver family that is characterized by phosphatase-active domain and C-terminal prenylation motif.[18] Overexpression of PRL-3 has been found in multiple cancers, including colorectal cancer, gastric cancer, liver cancer, and lung cancer.[19,20] It has been found to be associated with metastasis and proposed as a potential biomarker for assessing tumor aggressiveness and predicting outcomes of tumor therapy.[21,22] A recent study reported that the expression of PRL-3 is related to the clinical stages and recurrence of endometriosis.[23] Thus, it is interesting to explore whether there is an interaction between estrogen/P and PRL-3 during endometriosis progression and the possible molecular mechanisms.

In the present study, we aimed to investigate the effects of E2, P, and IL-6 treatments on PRL-3 expression in ESCs isolated from patients with endometriosis, on the migratory capacity of these cells, and on the PTEN-AKT signaling pathway.

Materials and Methods

Antibodies and Reagents

Sodium orthovanadate (Sov, an inhibitor for protein tyrosine phosphatases [PTPs][24]), recombinant human IL-6 (11386000010), E2, P, and type I collagen were purchased from Sigma-Aldrich (St Louis, Missouri). Antibodies to human PRL-3, β-actin, p-AKT (Ser437), AKT, cytokeratin 19 (CK19), and vimentin were purchased from Proteintech (Chicago, Illinois). Rabbit antihuman IL-6 (ab9324) and phosphatase and tensin homolog (PTEN, ab154812) antibodies were purchased from Abcam (Cambridge, Massachusetts). Horseradish peroxidase-conjugated goat antirabbit immunoglobulin G (IgG) was purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, California). Enhanced chemiluminescence (ECL) and Pierce bicinchoninic acid (BCA) kits were purchased from Thermo Fisher Scientific (Waltham, Massachusetts). Total RNA Purification Plus Kit was purchased from Norgen Biotek Corp (Thorold, Ontario, Canada). The SuperScript III First-Strand Synthesis System was purchased from Invitrogen (Life Technologies, Carlsbad, California), and the SYBR Green PCR Master Mix was purchased from Applied Biosystems (Foster City, California).

Collection of Endometriotic Tissues

This study was approved by the ethics committee of The Second Xiangya Hospital of Central South University. Samples of eutopic endometrium and ectopic endometrium from patients with endometriosis were collected from 20 patients who underwent resection of endometriotic cysts (chocolate cysts) via advanced laparoscopic surgery at the Department of Obstetrics and Gynecology of The Second Xiangya Hospital between May 2013 and January 2014. According to the revised American Fertility Society classification of endometriosis, the stages of endometriosis in these patients were stage II in 2 patients, stage III in 5 patients, and stage IV in 13 patients. All patients displayed a regular menstrual cycle, without medical complications or hormone treatment within 3 months prior to sample collection. For histology, the fresh tissues were fixed in 10% formalin solution, dehydrated in a graded ethanol series, and embedded in paraffin. Informed consent was obtained from all patients.

Isolation, Culture, and Treatment of ESCs

For cell isolation, fresh ovarian endometrial cysts from 6 patients (4 at the proliferative phase and 2 at the secretary phase of endometrium) were cut into small pieces using scissors and digested in 4 to 5 mL Dulbecco modified Eagle medium (DMEM; Invitrogen) containing 0.1% collagenase I for 20 to 30 minutes at 37°C. The cell mixture was centrifuged at 1000 rpm for 5 minutes, and the supernatant was discarded. The cell pellet was suspended in DMEM containing 10% fetal bovine serum (FBS) and cultured in an incubator with 5% CO_2 at 37°C. The identification of isolated cells was carried out by staining of CK19 and vimentin as previously described.[25]

For cell treatment, the ESCs were seeded into 6-well culture dishes at a density of 5×10^5 cells/mL. The complete culture medium was replaced by serum-free medium after 24 hours in culture. The cells were treated with 10^{-6} mol/L E_2,[14] 10^{-5} mol/L P,[26] 1.0 ng/mL IL-6,[27] 10^{-6} mol/L E2 + 1.0 ng/mL IL-6, 10^{-5} mol/L P + 1.0 ng/mL IL-6, E2 10^{-6} mol/L + 20 μg/mL IgG, or E2 10^{-6} mol/L + 20 μg/mL anti-IL-6 for 24 hours, and then the total mRNA and protein from the cells were extracted using conventional protocols. For the detection of PTEN, p-AKT, and AKT, the cells were cultured in complete culture medium for 24 hours and treated with 10^{-6} mol/L E2 + dimethyl sulfoxide (DMSO), 10^{-6} mol/L E2 + 0.5 μmol/L

Sov, 10^{-6} mol/L E2 + 1.0 ng/mL IL-6 + DMSO, or 10^{-6} mol/L E2 + 1.0 ng/mL IL-6 + 0.5 μmol/L Sov in serum-free medium for 24 hours. Total protein was isolated to assess the expression of PTEN, p-AKT, and AKT by Western blot analysis.

Scratch Wound Assay

The ESCs were seeded into 6-well culture dishes at a density of 5×10^5 cells/mL and grown to 100% confluence. A thin "wound" was made by scratching the cell layer with a 200-μL pipette tip, and the cells were washed 3 times with phosphate-buffered saline (PBS) to remove unattached cells. The cells were then treated with 10^{-6} mol/L E2 + DMSO, 0.5 μmol/L Sov, 10^{-6} mol/L E2 + 0.5 μmol/L Sov, 10^{-6} mol/L E2 + 1.0 ng/mL IL-6 + DMSO, or 10^{-6} mol/L E2 + 1.0 ng/mL IL-6 + 0.5 μmol/L Sov in DMEM containing 2% FBS for 36 hours. Representative images were captured before (0 hour) and after (36 hours) treatment. The width of the "wound" before and after treatment was measured using Image-Pro Plus (Media Cybernetics, Inc, Rockville, Maryland) to calculate the migration distance.

Real-Time Quantitative Reverse Transcriptase Polymerase Chain Reaction Analysis

Total RNA was extracted from ESCs using the Total RNA Purification Plus Kit following the manufacturer's instructions. A total of 1.0 μg RNA was reverse transcribed using the SuperScript III First-Strand Synthesis System following the manufacturer's protocol. All polymerase chain reaction (PCR) primers were synthesized by Sango Biotech (Shanghai, China), and the primer sequences were as follows: PRL-3-F (gene ID: 11156): 5′-AACGCCACGCTCAGCACCTTC-3′, PRL-3-R (gene ID: 11156): 5′-GGCTCAGCCAGTCTTCCACTACCTT-3′, β-actin-F: 5′-CATCCTGCGTCTGGACCTGG-3′, and β-actin-R: 5′-TAATGTCACGCACGATTTCC-3′. Each PCR was carried out using an ABI 7500 Detection System with Power SYBR (Applied Biosystems) with the following conditions: 95°C for 10 minutes, 40 cycles of 95°C for 10 seconds, 59°C for 50 seconds, and 72°C for 1 minute. To confirm the specificity of quantitative PCR (qPCR), dissociation curves were analyzed at the end of each qPCR assay. β-actin was used as a reference gene for normalization. The relative PRL-3 mRNA level was calculated by $2^{(\Delta Ct\cdot\text{Test} - \Delta Ct\cdot\text{Control})}$. The *Ct* of each target gene was compared to the *Ct* of the internal control β-actin gene.

Western Blot Analysis

The ESCs were lysed in 50 μL lysis buffer for 30 minutes and centrifuged at 14 000 rpm for 10 minutes to collect the supernatant. The protein concentration was determined using the BCA protein assay. A total of 60 mg protein was separated on 12% (for p-AKT) and 15% (for PRL-3) sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels in reducing condition and transferred onto 0.2-μm nitrocellulose membranes (Proteintech). The membrane was blocked in 5% nonfat milk in Tris-buffered saline containing Tween 20 for 1 hour and incubated with the appropriate primary antibody (PRL-3, 1:250 dilution; p-AKT and AKT, 1:500 dilution; PTEN, 1:1000; β-actin, 1: 2000) at 4°C overnight. After 3 washes, the membrane was incubated with the appropriate conjugated secondary antibody for 1 hour at room temperature and then exposed to reveal target protein bands using the ECL kit. The band intensity was quantitated using Quantity One software (Bio-Rad, Hercules, California).

Immunohistochemical Staining

Paraffin-embedded tissue sections cut at 5-μm thickness were prepared and heated at 68°C for 20 minutes. The sections were deparaffinized in xylene (5 minutes twice) and rehydrated in graded ethanol solutions (100%, 100%, 95%, 75%, and 50%, 5 minutes each). Antigen retrieval was performed using EDTA solution. The sections were treated with 3% H_2O_2 for 10 minutes to quench endogenous peroxidase activity. The sections were blocked with nonimmune goat serum to inhibit nonspecific binding and then incubated with rabbit antihuman PRL-3 antibody (1:600 dilution) or PBS (negative control) at 4°C overnight. After 3 washes in PBS, the sections were incubated with biotin-conjugated goat antirabbit secondary antibody for 30 minutes at room temperature and then stained with a 3,3′-diaminobenzidine kit and hematoxylin for counterstaining. The staining results were analyzed by light microscopy and scored by an experienced pathologist. The relative staining intensity (score 1: weak staining, straminеous; score 2: moderate staining, buffy; score 3: strong staining, brown) and percentage of positive cells in 5 random fields were each graded, and the results were multiplied. H-score is calculated by multiplying the intensity score with the percentage of positive cells.

Statistical Analysis

All data are presented as mean ± standard deviation values and analyzed using SPSS software version 17.0 (SPSS, Inc, Chicago, Illinois). All experiments included 6 individual samples ($n = 6$) and were repeated 3 times. The *t* test was used to evaluate differences between the 2 groups (before and after treatment), and 1-way analysis of variance was used to analyze differences among multiple groups. $P < .05$ was considered statistically significant.

Results

Overexpression of PRL-3 in Ectopic Endometrium

A previous study demonstrated that PRL-3 expression is associated with the clinical stages and recurrence of endometriosis,[23] suggesting that PRL-3 may be involved in this disease. Here, we compared the expression of PRL-3 protein in ectopic and entopic endometrium by immunohistochemistry. We

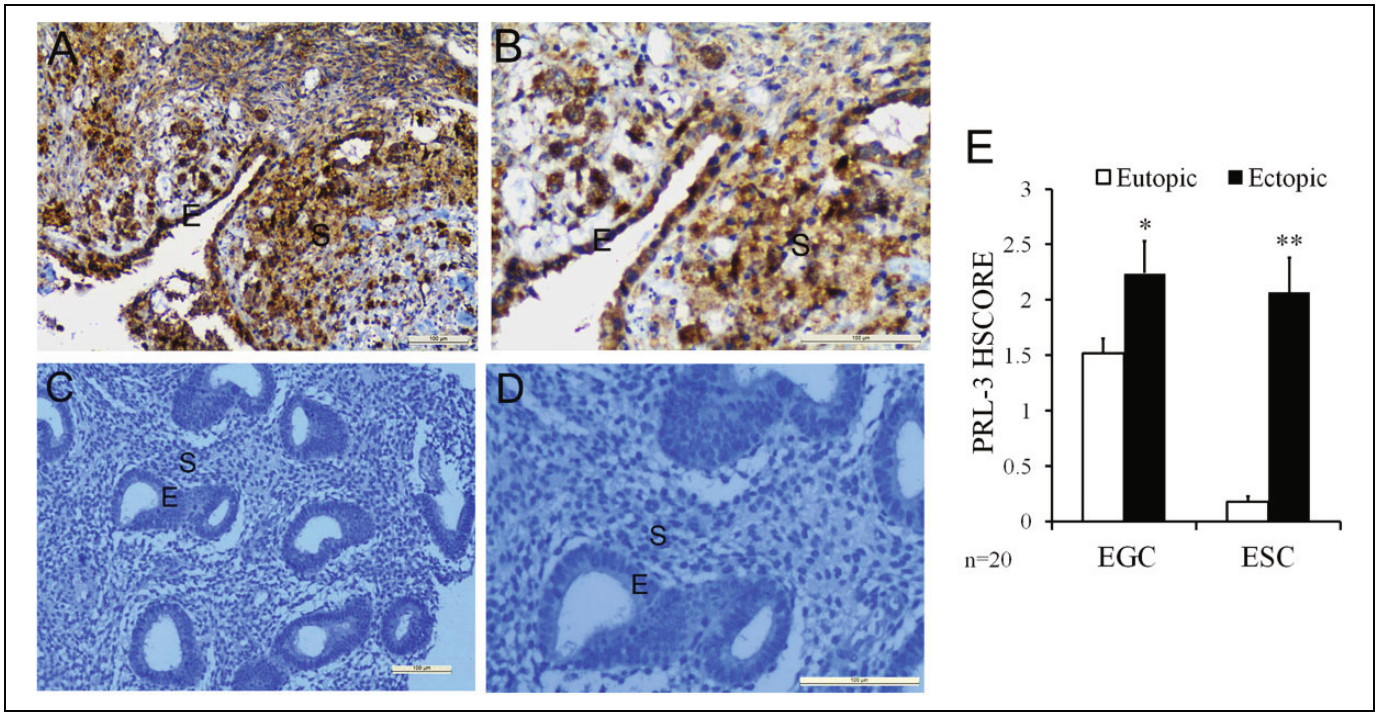

Figure 1. The expression of PRL-3 protein in ectopic and eutopic endometrium. A and B, Representative images of PRL-3 staining in ectopic endometrium (in the proliferative phase). A, ×100; B, ×200. C and D, Representative image of PRL-3 staining in eutopic endometrium (in the proliferative phase). C, ×100; D, ×200. Scale bar = 100 μm. E, Statistical analysis of PRL-3 staining in ectopic and eutopic endometrium. n = 20, *$P < .05$, **$P < .01$. EGCs indicates endometrial glandular cells; ESCs, endometrial stromal cells; PRL-3, phosphatase of regenerating liver 3.

observed that PRL-3 was highly expressed in the cytoplasm and membrane of endometrial glandular cells (EGCs) and ESCs in the ectopic endometrium (Figure 1A and B) but weakly expressed in the glandular epithelium and more weakly in the ESCs from eutopic endometrium (Figure 1C and D). Moreover, the expression levels of PRL-3 in EGCs and ESCs from ectopic endometrium were significantly higher than those in EGCs and ESCs from eutopic endometrium ($P < .05$ or $P < .01$; Figure 1E), whereas the levels of PRL-3 in the EGCs and ESCs were not significantly different from those in the ectopic endometrium ($P > .05$; Figure 1E).

Modulation of PRL-3 Expression in ESCs From Ectopic Endometrium by E2, P, and IL-6

Because endometriosis is a hormone-dependent disease, we sought to determine whether overexpression of PRL-3 in ectopic endometrium is mediated by hormone levels. Indeed, PRL-3 expression at both the mRNA and protein levels in ESCs from ectopic endometrium was significantly upregulated by E2 treatment ($P < .01$), whereas its mRNA and protein expression was inhibited by P treatment ($P < .05$; Figure 2A and B). Moreover, PRL-3 expression in ESCs was significantly elevated after IL-6 treatment ($P < .01$; Figure 2A and B). Interestingly, compared with E2 or IL-6 treatment alone, PRL-3 expression in ESCs was further increased upon combined treatment with E2 and IL-6 ($P < .05$ or $P < .01$; Figure 2A and B). However, the reduced PRL-3 expression in ESCs by P treatment was rescued by IL-6 treatment (Figure 2A and B).

Interleukin 6 Antibody Blocks the Effect of E2 on PRL-3 Expression in ESCs

Circulating cytokine levels are influenced by ovarian stimulation, and it has been reported that peak serum levels of IL-6, MCP-1, and paraoxonase 1 in women with endometriosis, polycystic ovarian syndrome, or unexplained infertility are positively correlated with E2 peak levels.[28] Here, we found that treatment with IL-6 antibody could significantly block the increase in PRL-3 mRNA and protein expression in ESCs induced by E2 treatment ($P < .05$; Figure 3A and B).

Effects of E2, IL-6, and PRL-3 on ESC Migration From Ectopic Endometrium

Endometrial stromal cell migration was evaluated by a scratch wound assay. Compared with the migration of control ESCs, E2 treatment significantly increased the migration of ESCs from ectopic endometrium, and combined treatment with E2 and IL-6 further enhanced the migration of ESCs ($P < .05$ or $P < .01$; Figure 4A and B). Interestingly, Sov treatment alone did not influence the migration of ESCs compared with that of control ESCs; however, Sov treatment significantly blocked the elevated migration of E2- and E2 + IL-6–treated ESCs ($P < .01$; Figure 4A and B).

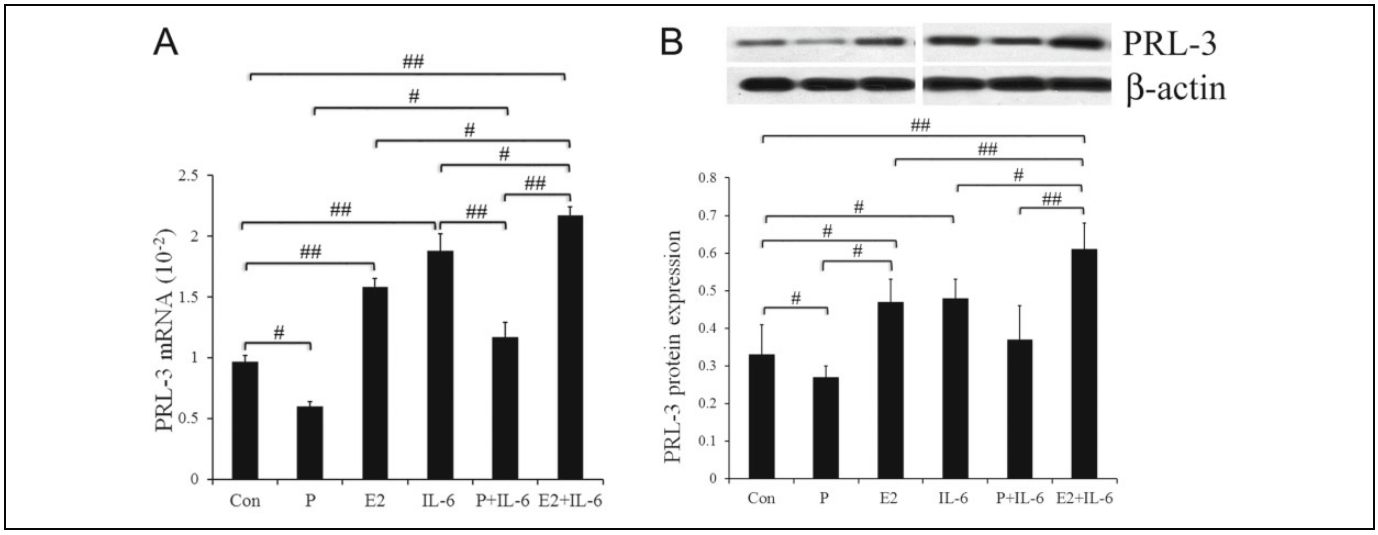

Figure 2. Effects of E2, P, and IL-6 on the expression of PRL-3 mRNA (A) and protein (B) in ESCs from ectopic endometrium. The isolated ESCs were treated with 10^{-5} mol/L P, 10^{-6} mol/L E_2, 1.0 ng/mL IL-6, 10^{-5} mol/L P + 1.0 ng/mL IL-6, or 10^{-6} mol/L E2 + 1.0 ng/mL IL-6 for 24 hours. Control cells received no treatment. Relative PRL-3 mRNA and protein expression is presented with normalization to β-actin. Significance was determined by 1-way ANOVA based on the results from 6 individual patients (n = 6). ANOVA indicates analysis of variance; ESCs, endometrial stromal cells; E2, 17β-estradiol; IL-6, interleukin 6; mRNA, messenger RNA; P, progesterone; PRL-3, phosphatase of regenerating liver 3.

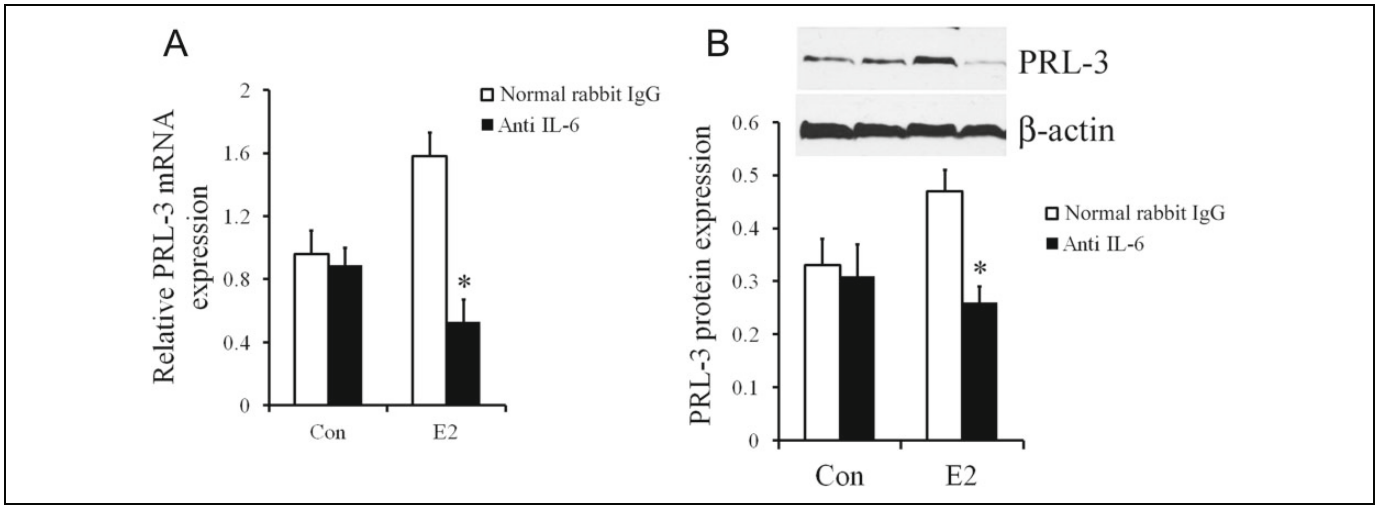

Figure 3. Effect of IL-6 antibody on E2-induced PRL-3 mRNA (A) and protein (B) expression in ESCs from ectopic endometrium. Relative PRL-3 mRNA and protein expression is presented with normalization to β-actin expression. n = 6, *$P < .05$. E2 indicates 17β-estradiol; ESCs, endometrial stromal cells; IL-6, interleukin 6; mRNA, messenger RNA; PRL-3, phosphatase of regenerating liver 3.

Effects of E2, IL-6, and PRL-3 on PTEN-AKT Signaling in ESCs From Ectopic Endometrium

A recent study reported that dysfunction of PTEN-PI3K/AKT signaling is associated with endometriosis.[29] Moreover, it has been demonstrated that estradiol may downregulate PTEN activity by increasing its phosphorylation[30] and PRL-3 can downregulate PTEN expression and promote epithelial–mesenchymal transition (EMT) through PI3K.[31] Consistent with previous findings, here we observed that the PTEN level was low in E2-treated ESCs, whereas the combined treatment with E2 and IL-6 slightly but not significantly upregulated the PTEN level in ESCs compared with that in ESCs treated with E2 only (Figure 5A and B). Moreover, PRL-3 inhibition by Sov could significantly upregulate the PTEN level in E2-treated and E2 + IL-6–treated ESCs ($P < .05$; Figure 5A and B). Because PTEN is a well-recognized suppressor of the PI3K-AKT pathway,[32] we sought to examine the effects of E2, IL-6, and PRL-3 on the activation of AKT signaling. In contrast to the PTEN expression trend, the ratio of p-AKT/AKT, an indicator of AKT activation, was high in E2-treated and E2 + IL-6–treated ESCs (Figure 5A and C), whereas Sov treatment significantly inhibited AKT activation in E2-treated and E2 + IL-6–treated ESCs ($P < .01$; Figure 5A and C).

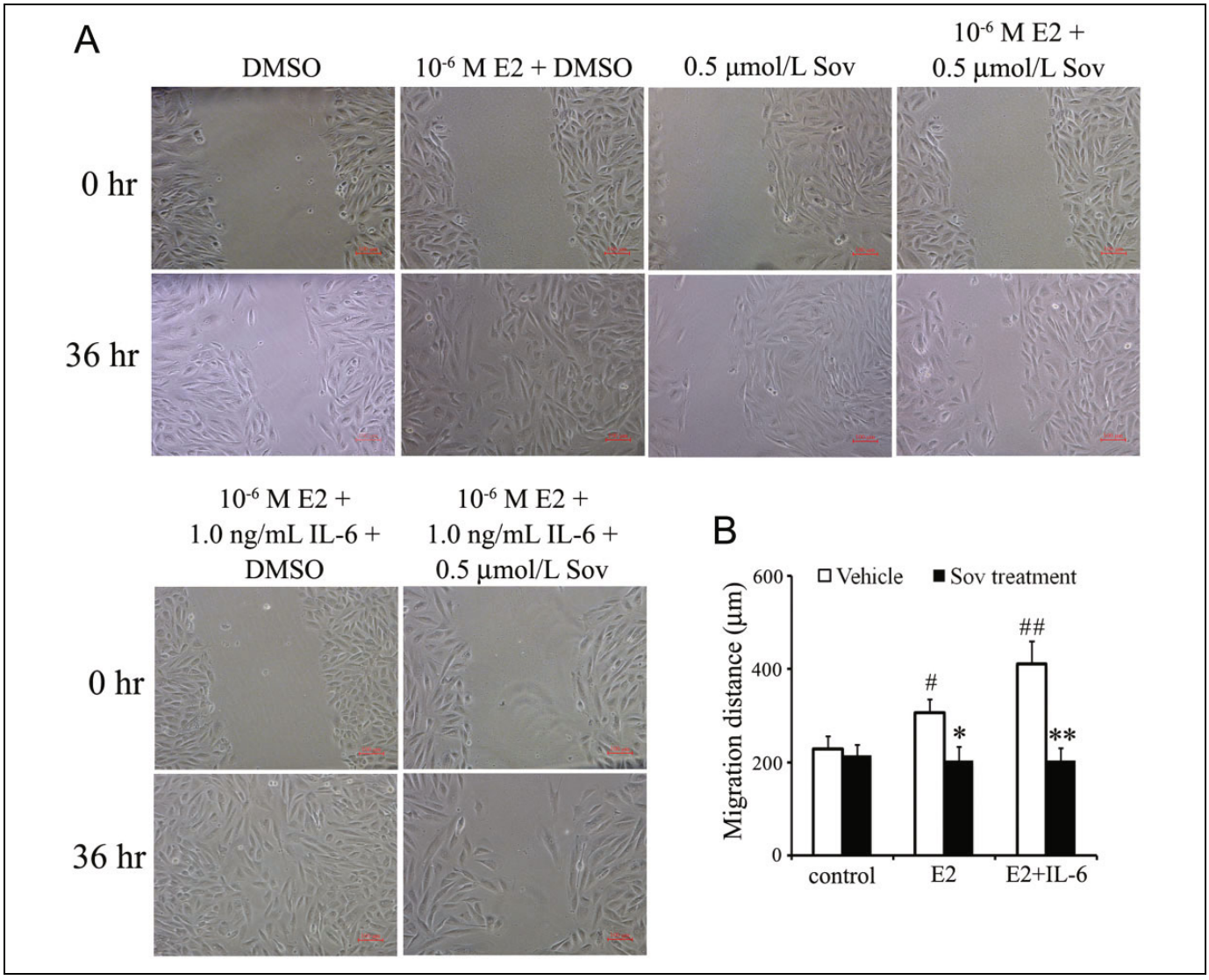

Figure 4. Effects of E2, IL-6, and PRL-3 on migration of ESCs from ectopic endometrium. A, Representative images of ESCs before and after treatment. Scale bar = 100 μm. B, Statistical analysis of cell migration after different treatments. Migration distance = width of gap at 0 hour − width of gap at 36 hours. n = 6. $^{\#}P < .05$, $^{\#\#}P < .01$, compared with DMSO control (between groups); $^{**}P < .01$, compared with DMSO (within E2 and E2 + IL-6 groups). DMSO indicates dimethyl sulfoxide; ESCs, endometrial stromal cells; E2 indicates 17β-estradiol; IL-6, interleukin 6; PRL-3, phosphatase of regenerating liver 3.

Discussion

Estrogen and P are 2 critical factors for maintaining endometrial function, because they regulate the expression of a large number of genes throughout the menstrual cycle.[33] Endometriosis is defined by the presence of estrogen-stimulated lesions, and both endogenous and exogenous estrogens have been found to regulate the development and progression of endometriosis.[2,8] Moreover, estrogen can increase the risk of laparoscopically visible endometriosis and the associated pelvic pain.[34] However, the role of P in the development of endometriosis has not been well understood. Endometriosis is a benign chronic inflammatory disorder. Estrogen and P exert direct effects on the production of cytokines and chemokines as well as the expression of local regulatory factors.[35] However, the molecular mechanisms underlying the roles of E2, P, and IL-6 in the development and progression of endometriosis have not been clearly clarified.

As a member of the PTPs family, PRL-3 has recently been identified as a marker for metastasis of malignant tumors and is associated with poor prognosis.[20,21] Previous studies have demonstrated that PRL-3 regulates cell migration and invasion in various cancers.[36] Moreover, Ruan et al reported that PRL-3 expression is mainly detected in endometriotic tissues but not in eutopic and normal endometrium, and PRL-3 expression is closely related to the clinical stages and recurrence of endometriosis.[23] Moreover, they demonstrated that PRL-3 knockdown by small interfering RNA can decrease the migratory capacity of ESCs, although no mechanistic data were presented.[37] Thus, we hypothesized that PRL-3 may play a specific role in the

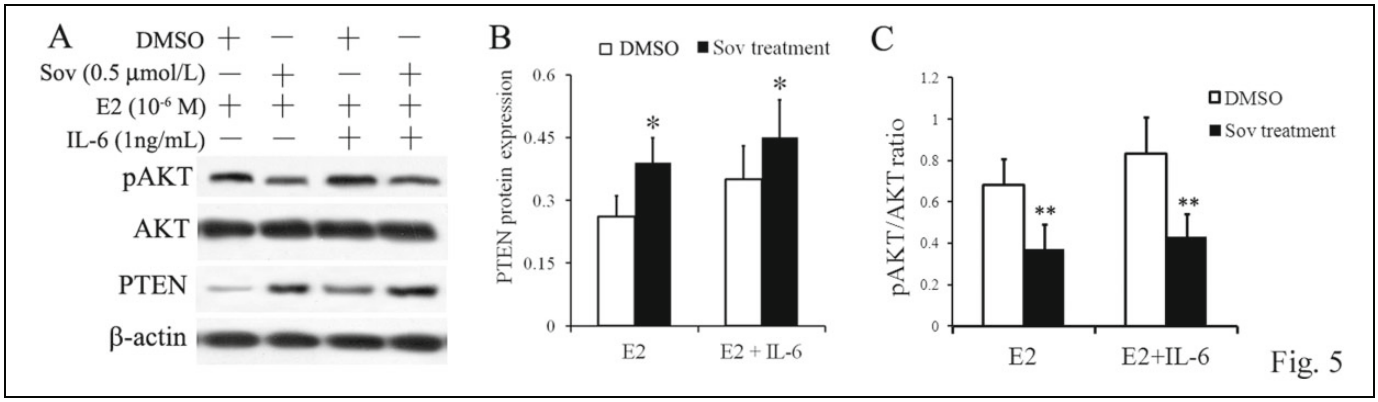

Figure 5. Effects of E2, IL-6, and PRL-3 on PTEN-AKT signaling pathway in ESCs from ectopic endometrium. A, Detection of PTEN, p-AKT, and AKT expression in ESCs after treatment by Western blot analysis. β-actin was used as an internal control. B, Statistical analysis for PTEN level. C, Statistical analysis for the ratio of p-AKT/AKT, phosphorylation protein kinase/protein kinase. n = 6, *$P < .05$; **$P < .01$. E2 indicates 17β-estradiol; ESCs, endometrial stromal cells; IL-6, interleukin-6; PTEN, phosphatase and tensin homolog.

migration of ESCs during endometriosis. Consistent with the results of a previous study,[23] we observed that PRL-3 was highly expressed in the EGCs and ESCs of ectopic endometrium. However, we observed weak expression of PRL-3 in the EGCs of eutopic endometrium, and this difference may have been caused by the sensitivity of the antibody. Thus, our data suggest that PRL-3 may be involved in mediating the pathogenesis of endometriosis.

Considering the important roles of E2 and P in the development and progression of endometriosis, we speculated that an interaction may exist between reproductive hormones such as E2 and P and PRL-3 during endometriosis. Indeed, we found that the expression of PRL-3 mRNA and protein in ESCs was upregulated by E2 treatment but downregulated by P treatment. These data provide the first evidence supporting the notion that PRL-3 is involved in the regulatory functions of E2 and P during endometriosis. Moreover, PRL-3 mRNA and protein expression levels in ESCs were elevated by IL-6 treatment and further increased upon combined treatment with E2 and IL-6. Furthermore, the enhanced PRL-3 expression induced by E2 treatment was completely reversed by treatment with IL-6 antibody. Previous studies reported that the serum IL-6 level is elevated in patients with endometriosis and E2 treatment significantly increases the secretion of IL-6 and TNF-α from peritoneal macrophages isolated from patients with endometriosis.[16,17] These data indicate that IL-6 is an important effector in the downstream signaling of E2 and is also an important intermediate player between E2 and PRL-3 expression. Interestingly, IL-6 treatment neutralized the inhibiting effect of P on PRL-3 expression in ESCs. Because there is no direct evidence supporting the ability of IL-6 to neutralize the effect of P, we speculate that P and IL-6 have opposite effects on PRL-3 expression via different molecular mechanisms, which results in overlapping effects on PRL-3 expression.

Although no evidence supports regulation of ESC migration by IL-6, it has been found that ovarian steroids significantly affect the migration of ESCs derived from women with endometriosis.[38] Gentilini et al observed that E2 (10^{-6}-10^{-8} mol) can stimulate the motility of ESCs by inducing cytoskeletal reorganization, whereas P pretreatment (10^{-7} mol) could not inhibit the migratory response induced by platelet-derived growth factor (PDGF-BB).[38] Here, we observed that both E2 and E2 + IL-6 treatments significantly increased the migratory capacity of ESCs isolated from ectopic endometrium. However, the elevated migratory capacity by E2 and E2 + IL-6 was completely abrogated by Sov, which can inhibit PRL-3 function. These data further verify that both E2 and IL-6 regulate functions such as the migration of ESCs during endometriosis via PRL-3, although the precise mechanisms require further investigation.

A previous study demonstrated that estrogen and growth factors can regulate the migration of normal ESCs via rapid activation of the ERK1/2 and PI3K/AKT intracellular signaling cascades.[14] Moreover, it has been reported that estradiol can downregulate PTEN activity by increasing its phosphorylation,[30] and PRL-3 downregulates PTEN expression and promotes EMT via PI3K/AKT signaling,[31] suggesting that PTEN/AKT signaling may be involved in E2- and IL-6–induced ESC migration via PRL-3. Indeed, the Sov upregulated the expression of PTEN but inhibited the activation of AKT signaling (reduced ratio of p-AKT/AKT) in E2-treated and E2 + IL-6–treated ESCs. Our results support our hypothesis that PRL-3 regulates the migratory potential of ESCs via downregulating PTEN expression, and a reduction in PTEN expression leads to the activation of AKT signaling. Furthermore, the regulatory roles of E2 and IL-6 in the development and progression of endometriosis involve PRL-3 and PTEN/AKT signaling.

In summary, our results demonstrate that E2 and IL-6 play key roles in the migratory potential of ESCs in the ectopic endometrium via upregulation of PRL-3 expression, which is involved in the PTEN-AKT signaling pathway.

Acknowledgments

The authors would like to thank the Department of Obstetrics and Gynecology of the Second Xiangya Hospital for providing the samples.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Quaas AM, Weedin EA, Hansen KR. On-label and off-label drug use in the treatment of endometriosis. *Fertil Steril*. 2015;103(3): 612-625.
2. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril*. 2012;98(3):511-519.
3. Cramer DW, Missmer SA. The epidemiology of endometriosis. *Ann N Y Acad Sci*. 2002;955:11-22; discussion 34-16, 396-406.
4. Gao X, Outley J, Botteman M, Spalding J, Simon JA, Pashos CL. Economic burden of endometriosis. *Fertil Steril*. 2006;86(6): 1561-1572.
5. Bulun SE. Endometriosis. *N Engl J Med*. 2009;360(3):268-279.
6. Nezhat F, Datta MS, Hanson V, Pejovic T, Nezhat C, Nezhat C. The relationship of endometriosis and ovarian malignancy: a review. *Fertil Steril*. 2008;90(5):1559-1570.
7. Van Gorp T, Amant F, Neven P, Vergote I, Moerman P. Endometriosis and the development of malignant tumours of the pelvis. A review of literature. *Best Pract Res Clin Obstet Gynaecol*. 2004; 18(2):349-371.
8. Shao R, Cao S, Wang X, Feng Y, Billig H. The elusive and controversial roles of estrogen and progesterone receptors in human endometriosis. *Am J Transl Res*. 2014;6(2):104-113.
9. Brandenberger AW, Lebovic DI, Tee MK, et al. Oestrogen receptor (ER)-alpha and ER-beta isoforms in normal endometrial and endometriosis-derived stromal cells. *Mol Hum Reprod*. 1999; 5(7):651-655.
10. Cavallini A, Resta L, Caringella AM, Dinaro E, Lippolis C, Loverro G. Involvement of estrogen receptor-related receptors in human ovarian endometriosis. *Fertil Steril*. 2011;96(1): 102-106.
11. Bergqvist A, Ferno M, Skoog L. Quantitative enzyme immunoassay and semiquantitative immunohistochemistry of oestrogen and progesterone receptors in endometriotic tissue and endometrium. *J Clin Pathol*. 1997;50(6):496-500.
12. Nisolle M, Casanas-Roux F, Wyns C, de Menten Y, Mathieu PE, Donnez J. Immunohistochemical analysis of estrogen and progesterone receptors in endometrium and peritoneal endometriosis: a new quantitative method. *Fertil Steril*. 1994;62(4):751-759.
13. Zeitoun K, Takayama K, Michael MD, Bulun SE. Stimulation of aromatase P450 promoter (II) activity in endometriosis and its inhibition in endometrium are regulated by competitive binding of steroidogenic factor-1 and chicken ovalbumin upstream promoter transcription factor to the same cis-acting element. *Mol Endocrinol*. 1999;13(2):239-253.
14. Gentilini D, Busacca M, Di Francesco S, Vignali M, Vigano P, Di Blasio AM. PI3K/Akt and ERK1/2 signalling pathways are involved in endometrial cell migration induced by 17beta-estradiol and growth factors. *Mol Hum Reprod*. 2007;13(5): 317-322.
15. Bersinger NA, Dechaud H, McKinnon B, Mueller MD. Analysis of cytokines in the peritoneal fluid of endometriosis patients as a function of the menstrual cycle stage using the Bio-Plex(R) platform. *Arch Physiol Biochem*. 2012;118(4): 210-218.
16. Agic A, Xu H, Finas D, Banz C, Diedrich K, Hornung D. Is endometriosis associated with systemic subclinical inflammation? *Gynecol Obstet Invest*. 2006;62(3):139-147.
17. Khan KN, Kitajima M, Inoue T, Fujishita A, Nakashima M, Masuzaki H. 17beta-estradiol and lipopolysaccharide additively promote pelvic inflammation and growth of endometriosis. *Reprod Sci*. 2015;22(5):585-594.
18. Kozlov G, Cheng J, Ziomek E, Banville D, Gehring K, Ekiel I. Structural insights into molecular function of the metastasis-associated phosphatase PRL-3. *J Biol Chem*. 2004;279(12): 11882-11889.
19. Saha S, Bardelli A, Buckhaults P, et al. A phosphatase associated with metastasis of colorectal cancer. *Science*. 2001;294(5545): 1343-1346.
20. Miskad UA, Semba S, Kato H, Yokozaki H. Expression of PRL-3 phosphatase in human gastric carcinomas: close correlation with invasion and metastasis. *Pathobiology*. 2004;71(4): 176-184.
21. Li ZR, Wang Z, Zhu BH, et al. Association of tyrosine PRL-3 phosphatase protein expression with peritoneal metastasis of gastric carcinoma and prognosis. *Surg Today*. 2007;37(8):646-651.
22. Wang Z, He YL, Cai SR, et al. Expression and prognostic impact of PRL-3 in lymph node metastasis of gastric cancer: its molecular mechanism was investigated using artificial microRNA interference. *Int J Cancer*. 2008;123(6):1439-1447.
23. Ruan F, Lin J, Wu RJ, et al. Phosphatase of regenerating liver-3: a novel and promising marker in human endometriosis. *Fertil Steril*. 2010;94(6):1980-1984.
24. Wu X, Zeng H, Zhang X, et al. Phosphatase of regenerating liver-3 promotes motility and metastasis of mouse melanoma cells. *Am J Pathol*. 2004;164(6):2039-2054.
25. Ryan IP, Schriock ED, Taylor RN. Isolation, characterization, and comparison of human endometrial and endometriosis cells in vitro. *J Clin Endocrinol Metab*. 1994;78(3):642-649.
26. Akoum A, lemay A, Paradis I, Rheault N, Maleux R. Secretion of interleukin-6 by human endometriotic cells and regulation by proinflammatory cytokines and sex steroid. *Hum Reprod*. 1996; 11(10):2269-2275.
27. Shain KH, Yarde DN, Meads MB, et al. Beta1 integrin adhesion enhances IL-6-mediated STAT3 signaling in myeloma cells: implications for microenvironment influence on tumor survival and proliferation. *Cancer Res*. 2009;69(3):1009-1015.
28. Younis A, Hawkins K, Mahini H, Butler W, Garelnabi M. Serum tumor necrosis factor-alpha, interleukin-6, monocyte chemotactic protein-1 and paraoxonase-1 profiles in women with endometriosis, PCOS, or unexplained infertility. *J Assist Reprod Genet*. 2014;31(11):1445-1451.
29. Govatati S, Kodati VL, Deenadayal M, Chakravarty B, Shivaji S, Bhanoori M. Mutations in the PTEN tumor gene and risk of

endometriosis: a case-control study. *Hum Reprod*. 2014;29(2): 324-336.
30. Guzeloglu-Kayisli O, Kayisli UA, Al-Rejjal R, Zheng W, Luleci G, Arici A. Regulation of PTEN (phosphatase and tensin homolog deleted on chromosome 10) expression by estradiol and progesterone in human endometrium. *J Clin Endocrinol Metab*. 2003; 88(10):5017-5026.
31. Wang H, Quah SY, Dong JM, Manser E, Tang JP, Zeng Q. PRL-3 downregulates PTEN expression and signals through PI3K to promote epithelial–mesenchymal transition. *Cancer Res*. 2007; 67(7):2922-2926.
32. Georgescu MM. PTEN Tumor Suppressor Network in PI3K-Akt Pathway Control. *Genes Cancer*. 2010;1(12):1170-1177.
33. Kao LC, Tulac S, Lobo S, et al. Global gene profiling in human endometrium during the window of implantation. *Endocrinology*. 2002;143(6):2119-2138.
34. Kim JJ, Kurita T, Bulun SE. Progesterone action in endometrial cancer, endometriosis, uterine fibroids, and breast cancer. *Endocr Rev*. 2013;34(1):130-162.
35. Weiss G, Goldsmith LT, Taylor RN, Bellet D, Taylor HS. Inflammation in reproductive disorders. *Reprod Sci*. 2009;16(2):216-229.
36. Lee SK, Han YM, Yun J, et al. Phosphatase of regenerating liver-3 promotes migration and invasion by upregulating matrix metalloproteinases-7 in human colorectal cancer cells. *Int J Cancer*. 2012;131(3):E190-E203.
37. Ruan F, Wu RJ, Xu KH, et al. Effects of PRL-3 siRNA on the migration of endometriotic stromal cells. *Zhonghua Yi Xue Za Zhi*. 2011;91(23):1613-1616.
38. Gentilini D, Vigano P, Somigliana E, et al. Endometrial stromal cells from women with endometriosis reveal peculiar migratory behavior in response to ovarian steroids. *Fertil Steril*. 2010;93(3): 706-715.