Regulation of P21-Activated Kinase-4 by Progesterone and Tumor Necrosis Factor-α in Human Endometrium and Its Increased Expression in Advanced-Stage Endometriosis

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Context: Endometriosis is a common gynecological condition characterized by enhanced proliferation, adhesiveness, invasiveness, and survival of endometrial cells after retrograde menstruation. Originally identified as a cytoskeletal regulatory kinase, p21-activated kinase 4 (Pak4) regulates diverse cellular activities that might be altered in the establishment and progression of endometriosis.

Objective: The aim was to evaluate the effects of sex steroids and proinflammatory cytokines on the Pak4 expression in endometrial cells along with the functional change caused by inhibition of Pak4 expression as well as to see whether the Pak4 expression is altered in endometriosis.

Methods: Pak4 expression was analyzed using immunohistochemistry and Western blot analysis. Viability and invasiveness were assayed after transfection of endometrial cells with Pak4 small interfering RNA.

Results: The Pak4 expression was significantly decreased in the stromal cells during the secretory phase as well as by in vitro treatment with progesterone. The immunoreactivity of Pak4 was significantly increased in the eutopic endometrium as well as in the ovarian endometriotic cyst of women with endometriosis compared with the control subjects. TNF-α induced a significant increase in the Pak4 expression in endometrial cells in vitro, whereas IL-1β had no effects. Transfection of endometrial cells with Pak4 small interfering RNA led to a significant decrease in viability and invasiveness in endometrial cells.

Conclusion: These findings suggest that Pak4 is regulated by progesterone and TNF-α in endometrial cells and that the increased expression of Pak4 might lead to the establishment and progression of endometriosis by enhanced cellular viability and invasiveness in endometrial cells. (J Clin Endocrinol Metab 98: E238–E248, 2013)
adhesion (8, 9). Activation of Pak4 provides resistance to apoptosis and promotes cell survival, whereas cells with reduced Pak4 expression have an increased sensitivity to cell stress (9–11). Pak4 is the only Pak family member that is oncogenic when overexpressed, and cells overexpressing activated Pak4 exhibit a lack of anchorage-dependent growth that is comparable with Ras transformation (9, 12, 13). Indeed, Pak4 overexpression has been shown in a number of tumor cell lines, including breast, prostate, and gall bladder and in primary tumors (9). Furthermore, several studies have revealed a specific role for Pak4 in cancer cell migration and invasiveness (9, 14, 15).

Defined as the presence of endometrial tissue outside the uterus, endometriosis causes diverse diseases, including infertility, pelvic pain, and dysmenorrhea. Although the mechanisms responsible for its pathogenesis and progression remain poorly understood, endometriosis is characterized by enhanced proliferation, adhesiveness, invasiveness, and survival of endometrial cells after retrograde menstruation. Previously we have revealed that Pak1 is down-regulated in endometrial cells in vitro by progestin treatment and exhibits a decrease in expression during the secretory phase in normal endometrium (16). We also found that inhibition of Pak1 expression by Pak1 small interfering RNA (siRNA) transfection led to decreased viability of endometrial cells and showed that the expression of Pak1 is increased during the secretory phase in the eutopic endometrium of women with endometriosis as well as in the ovarian endometriotic cysts (16, 17).

Based on the emerging importance of Pak4 in cellular proliferation, adhesiveness, invasiveness, and survival, which all might be altered in the pathogenesis and/or progression of endometriosis, it seems necessary to evaluate whether the expression of Pak4 is increased in the eutopic endometria as well as in the ovarian endometriotic cysts (16, 17).

Materials and Methods

Tissue collection

For endometrial stromal cell (ESC) cultures, endometrial samples were obtained from fertile women diagnosed with intramural leiomyoma at the time of hysterectomy, who had no evidence of endometrial abnormalities, adenomyosis, or pelvic endometriosis and who had not taken any hormonal medication in the preceding 3 months. The endometrial samples were placed in Hanks’ balanced salt solution and transported to the laboratory for ESC isolation and culture. Written informed consents were obtained from each patient using consent forms and protocols approved by the Institutional Review Board for Human Research of Asan Medical Center.

For immunohistochemical staining, endometrial sections were obtained from a total of 38 women with histological evidence of endometriosis and those from 34 women with carcinoma in situ of the uterine cervix served as controls. All of the recruited women had regular menstrual cycles and had undergone hysterectomies by transabdominal or laparoscopic methods. Women with endometrial abnormality, adenomyosis, or pelvic endometriosis were excluded from the control group. All of the women in the endometriosis group were confirmed as having advanced-stage endometriosis: 13 had only endometriotic cysts, whereas 19 and 5 patients had concurrent leiomyomas and adenomyosis in the histological reports, respectively. In the endometriosis group, the extent of disease was staged according to the American Society for Reproductive Medicine (18). The date of the menstrual cycle were classified as proliferative (days 1–13), early secretory (days 14–19), midsecretory (days 20–23), and late secretory (days 24–28) phases by endometrial histology using the criteria of Noyes et al (19). Sections of endometriotic cyst were also obtained from 25 nulliparous women in whom ovarian cystectomy was performed due to ovarian endometrioma. Because both glandular and stromal structures were not always present in 1 section, we preselected only the sections containing both structures in the endometriotic cysts before immunohistochemistry. The clinical characteristics, the menstrual phases of each group, and the stages of endometriosis are summarized in Table 1. We did not need additional informed consent to use the specimens in this study because only archived material was used. The institutional review board for human research in our hospital approved this project.

Chemicals and cell lines

Estradiol (E2), progesterone (P), and Ishikawa cell line (a well differentiated endometrial adenocarcinoma cell line) were purchased from Sigma-Aldrich (St Louis, Missouri).

Isolation and culture of human ESCs

ESCs were separated and maintained in monolayer culture as described previously (16, 20). After isolation, ESCs were passed by standard methods of trypsinization, plated in culture dishes, and grown in DMEM supplemented with 10% charcoal-stripped calf serum (Flow Laboratories, Rockville, Maryland). ESCs after first passage were assayed immunocytochemically using specific cell surface markers, and we have previously shown that the purity of isolated ESCs was more than 95% (16, 21). We used only the cells after the first passage in all of the experiments using ESCs.

Experimental setup

Each experiment with Ishikawa cells was performed using cells prepared separately at 11 different times for sex steroid treatment and 5 different times for cytokines treatment. Each experiment with ESCs was performed using cells prepared from endometrial tissue specimens obtained from 14 different patients for sex steroid treatment and 10 different patients for cytokines treatment. When Ishikawa cells and ESCs were grown to 70%
confluence, they were treated with serum-free, phenol red-free media (Sigma-Aldrich) for 24 hours before treatment. To evaluate the effect of sex steroids on Pak4 expression, cell cultures were treated with vehicle (control), E2 \(10^{-8} \) M, or P \(10^{-7} \) M for 24 and 48 hours, respectively. To evaluate the effect of TNF-α or IL-1β on Pak4 expression, cell cultures were treated with vehicle (control), TNF-α (10 or 25 ng/mL), or IL-1β (10 or 25 ng/mL), respectively. The treatment dose and time of E2, P, TNF-α, and IL-1β were chosen based on the previous studies showing the effects of sex steroids or cytokines on Pak1 (16, 17).

**Western blot analysis**

Total protein extraction and the measurement of concentrations were performed as described in our previous study (16). The expression of Pak4 was evaluated by Western blot analysis as described previously (16) as well using primary antibody against Pak4 (rabbit polyclonal antihuman Pak4 antibody [Cell Signaling Technology, Beverly, Massachusetts]) and horseradish peroxidase-conjugated antirabbit secondary antibody (Vector Laboratories, Burlingame, California). Each experiment was repeated at least 3 times.

**Immunohistochemistry**

Formalin-fixed, paraffin-embedded samples were cut into 4-μm sections. After deparaffinization, immunohistochemical staining was performed as described in our previous study (16) using a rabbit polyclonal antihuman Pak4 antibody (Abcam, Cambridge, Massachusetts) as primary antibody. The intensity for Pak4 immunoreactivity was semiquantitatively evaluated and analyzed as described in our previous study as well (16).

**siRNA transfection**

Pak4 siRNA and nonspecific control siRNA were designed and produced by Genolution (Seoul, South Korea). Ishikawa cells and ESCs, grown to 30% confluence, were transfected using G-Fectin (Genolution) with Pak4 siRNA or nonspecific control siRNA according to the manufacturer’s instructions. The 21-mer sequence of Pak4 siRNA was 50-AACTTCATCAAGATAGGC-GAG-30 (forward) and 30-TTGAAGTATGTCTAACCCGTC-50 (reverse). The final concentration of siRNA was 30 nmol/L. Total proteins were extracted and Western blot analysis was performed to confirm protein level at 48 hours for Ishikawa cells or 72 hours for ESCs after transfection.

**High-sensitive water soluble tetrachlorphthalein salt cell viability assay**

Cell viability was assessed by an EZ-Cytox cell viability assay kit (Daeelab Service, Seoul, Korea). Cells transfected with Pak4 siRNA or nonspecific control siRNA were incubated for 48 hours for Ishikawa cells \((n = 5)\) or 72 hours for ESCs \((n = 4)\). Then cells were detached and seeded on 96-well plates with 5000 cells/well. Ten microliters of high-sensitive, water-soluble tetrachlorphthalein salt assay reagent were added to each well at day 2 for Ishikawa cells and day 3 for ESCs. The plates were incubated at 37°C for 1 hour. Cell viability was determined by measuring the absorbance of samples at 450 nm.

**Invasion assay**

Invasion assays were performed in a 96-well transwell containing 8-μm pore-size inserts (Corning, Corning, New York) coated with Basement Membrane Extract Cultrex (Trevigen, Gaithersburg, Maryland). Cells transfected with Pak4 siRNA or nonspecific control siRNA were incubated for 48 hours for Ishikawa cells \((n = 6)\) or 72 hours for ESCs \((n = 4)\). Then cells were starved in serum-free DMEM medium for 18 hours. Fifty microliters of cell suspension \((50,000 \text{ cells/well})\) were added to the top compartment and 100 μl of serum-free DMEM medium was added to the bottom compartment of the chamber. The chambers were incubated at 37°C in humidified air with 5% CO₂ for 24 hours. Then both compartments of the chambers were washed with washing buffer, and invaded cells were labeled with 5 μg/mL calcein-AM (Trevigen) in cell dissociation solution at 37°C for 1 hour. Cell viability was determined by measuring the absorbance of samples at 450 nm.

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**Table 1. Clinical Characteristics of Patients and Controls**

<table>
<thead>
<tr>
<th></th>
<th>Control Group ((n = 34))</th>
<th>Endometrosis-Hysterectomy Group ((n = 38))</th>
<th>Endometrioma-Cystectomy Group ((n = 25))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (^{a})</td>
<td>37.4 ± 3.7</td>
<td>39.1 ± 5.5</td>
<td>25.3 ± 5.7 (^{b})</td>
</tr>
<tr>
<td>Number of deliveries(^{a})</td>
<td>1.9 ± 0.6</td>
<td>1.2 ± 0.8(^{c})</td>
<td>0</td>
</tr>
<tr>
<td>Menstrual phases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proliferative</td>
<td>14(^{d})</td>
<td>15(^{d})</td>
<td>14(^{e})</td>
</tr>
<tr>
<td>Secretory</td>
<td>20(^{d})</td>
<td>23(^{d})</td>
<td>11(^{e})</td>
</tr>
<tr>
<td>Early secretory</td>
<td>4(^{d})</td>
<td>5(^{d})</td>
<td></td>
</tr>
<tr>
<td>Midsecretory</td>
<td>6(^{d})</td>
<td>8(^{d})</td>
<td></td>
</tr>
<tr>
<td>Late secretory</td>
<td>10(^{d})</td>
<td>10(^{d})</td>
<td></td>
</tr>
<tr>
<td>AFS Classification of endometriosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>26</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Serum CA-125 level(^{f})</td>
<td>71.8 ± 13.1</td>
<td>70.5 ± 16.6</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: AFS, American Fertility Society.

\(^{a}\) Values are mean ± SD.

\(^{b}\) \(P < .001\) vs the controls, derived by Student’s t test.

\(^{c}\) \(P < .05\) vs the controls, derived by Student’s t test.

\(^{d}\) Based on the histological dating.

\(^{e}\) Based on the menstrual history.

\(^{f}\) Values are mean ± SE (units per milliliter).
37°C for 1 hour. Cell invasion was determined by measuring the absorbance of samples at 485 nm excitation, 520 nm emission using same parameters (time and gain) with an Victor X3 multilabel plate reader (PerkinElmer, Waltham, Massachusetts).

Statistical analysis
All of the data were normally distributed as assessed by a Kolmogorov-Smirnov test. ANOVA and post hoc Tukey test for pairwise comparisons were used for statistical analysis for the Western blot analyses data on the effects of sex steroids and cytokines as well as the histological scores (HSCOREs). A Student t test was performed to compare the average between 2 groups in other data. Statistical analyses were performed using Statistical Programs for the Social Sciences software program (version 14.0; SPSS, Chicago, Illinois). Statistical significance was defined as $P < .05$.

Results

Effects of sex steroids on the expression of Pak4 in Ishikawa cells and ESCs

There were no significant differences of Pak4 expression in Ishikawa cells ($n = 11$) among different sex steroids treatment groups after 24 and 48 hours, respectively (Figure 1A). Although in vitro E2 or P treatment for 24 hours had no effect on Pak4 protein level in ESCs ($n = 14$), P treatment for 48 hours led to a significant decrease of Pak4 expression compared with the control group ($P < .005$) (Figure 1B).

Effects of cytokines on the expression of Pak4 in Ishikawa cells and ESCs

Ishikawa cells ($n = 5$) and ESCs ($n = 10$) treated with TNF-α (10 or 25 ng/ml) for 24 hours led to a significant increase of Pak4 expression compared with the controls ($P < .05$; $P < .05$, respectively) (Figure 2, Aa and Ba), whereas treatment with IL-1β (10 or 25 ng/ml) for 24 hours did not affect Pak4 level compared with the controls, respectively (Figure 2, Ab and Bb). After treatment for 48 hours, both TNF-α and IL-1β in Ishikawa cells (Figure 2, Ac and Ad) and ESCs (Figure 2, Bc and Bd) did not show a significant difference compared with the controls, respectively.

Expression of Pak4 in the endometrium of the control subjects

Pak4 immunoreactivity was cytoplasmic and intramembranous in endometrial glandular and stromal cells (Figure 3Aa). There was no difference in Pak4 immunoreactivity of the glandular cells between the proliferative phase and the secretory phase. However, the Pak4 expression was significantly decreased during the secretory phase compared with the proliferative phase in the stromal cells within the control subjects ($P < .005$) (Figure 3Ab).

Expression of Pak4 in the eutopic endometrium of women with endometriosis

The expression of Pak4 was also cytoplasmic and intramembranous in endometrial glandular and stromal cells in the eutopic endometrium of women with endometriosis. Including endometria for all menstrual phases put together, the immunoreactivity of Pak4 was significantly increased in both the glandular cells and the stromal cells of the eutopic endometrium of women with endometriosis compared with the control subjects ($P < .005$ and $P < .005$, respectively) (Figure 3B). The immunoreactivity of Pak4 was significantly increased in the glandular cells of the eutopic endometrium of women with endometriosis compared with the control subjects during the proliferative phase ($P < .005$) as well as the secretory phase ($P < .005$) (Figure 3Bc). The expression of Pak4 was also higher in the stromal cells of the eutopic endometrium of women with endometriosis compared with the control subjects during the proliferative phase ($P < .05$) as well as the secretory phase ($P < .005$), and the main increase was during the midsecretory phase ($P < .005$) (Figure 3Bd). The Pak4 expression in the stromal cells was not different between the proliferative and the secretory phase within the endometriosis group, whereas there was a significant decrease of Pak4 expression in the stromal cells during the secretory phase within the control subjects ($P < .001$, derived by ANOVA among 4 groups).

Expression of Pak4 in the ovarian endometriotic cysts

Based on the findings that Pak4 expression is higher in the eutopic endometrium of women with endometriosis compared with the controls and that TNF-α can also lead to increased expression of Pak4, we evaluated whether ovarian endometriotic cyst has higher Pak4 expression compared with the eutopic endometrium with and without endometriosis. The Pak4 immunoreactivity was higher in endometriotic cyst in glandular cells compared with the controls ($P < .001$) as well as the eutopic endometrium of women with endometriosis ($P < .001$) (Figure 3C). We could also see higher expression of Pak4 in the stromal cells of endometriotic cyst compared with the controls ($P < .001$), without obvious difference compared with the eutopic endometrium of women with endometriosis (Figure 3C). There was no difference in the Pak4 expression between the proliferative and secretory phases in the ovarian endometriotic cyst.
Cell viability and invasion assay after Pak4 siRNA transfection

To investigate the effect of Pak4 expression on the viability and invasiveness of endometrial cells, Ishikawa cells, and ESCs were transfected with Pak4 siRNA and nonspecific control siRNA. Transfection with Pak4 siRNA led to decrease of Pak4 expression to 27.8±6.4% level (mean ± SE, $P < .001$) in Ishikawa cells and 37.7±5.5% level ($P < .005$) in ESCs compared with those transfected with nonspecific control siRNA in experiments for viability assay (Figure 4). After transfection with Pak4 siRNA, cell viability was significantly reduced in Ishikawa cells ($P < .01$) and ESCs ($P < .05$) compared with those transfected with nonspecific control siRNA (Figure 4).

Transfection with Pak4 siRNA led to decrease of Pak4 expression to 39.0±14.2% level ($P < .01$) in Ishikawa...
cells and 35.4 ± 9.2% level (P < .01) in ESCs compared with those transfected with nonspecific control siRNA in experiments for invasion assay (Figure 5). After transfection with Pak4 siRNA, cell invasion was significantly reduced in Ishikawa cells (P < .001) as well as ESCs (P < .05) compared with those transfected with nonspecific control siRNA (Figure 5). It is possible that the decreased invasion was partly induced by the decreased cellular viability. However, considering that the cellular invasion was much more reduced (~80% in Ishikawa cells and ~82% in ESCs, Figure 5) than viability (~9% in Ishikawa cells and ~25% in ESCs, Figure 4), it is more likely that the invasion was further decreased with Pak4 inhibition, which is not totally dependent on the reduced viability.

**Discussion**

The present study evaluated the effects of sex steroids and proinflammatory cytokines on the Pak4 expression as well as the functional change caused by inhibition of Pak4 expression in endometrial cells. We also investigated whether the Pak4 expression is altered in the eutopic endometria and/or the ovarian endometriotic cysts of women with advanced stage endometriosis. We have demonstrated the following: 1) the Pak4 expression was significantly decreased in the stromal cells during the secretory phase as well as by in vitro P treatment; 2) TNF-α induced a significant increase in the Pak4 expression in endometrial cells in vitro; 3) transfection of endometrial cells with Pak4 siRNA led to a significant decrease in viability and invasiveness in endometrial cells; and 4) the immunoreactivity of Pak4 was significantly increased in the eutopic endometrium as well as in the ovarian endometriotic cyst of women with endometriosis compared with the control subjects.

Although the development of endometriosis has historically been viewed as an estrogen-dependent disease, several investigators have suggested that a failure of P to regulate the expression of genes during endometrial differentiation might be a critical component of the disease process (22–24). The present study showed a significant decrease of Pak4 expression in ESCs after in vitro P treat-
Figure 3. Representative micrographs (Aa) (×400) and HSCOREs (Ab) of Pak4 immunostaining in the endometrium of the control subjects. Representative micrographs of Pak4 immunostaining in the eutopic endometrium during the midsecretory phase (Ba, control subject; Bb, endometriosis patient) (×400) and HSCOREs in endometrial glandular (Bc) and stromal cells (Bd) throughout the menstrual cycle. Representative micrographs of Pak4 immunostaining in the endometrium of the control subjects (Ca) and the ovarian endometriotic cyst (Cb) (×400) and HSCOREs in the glandular (Cc) and stromal cells (Cd) throughout the menstrual cycle. EP, early proliferative phase; ES, early secretory phase; LP, late proliferative phase; LS, late secretory phase; MP, midproliferative phase; MS, midsecretory phase; N, negative control; P, proliferative phase; S, secretory phase; W, whole menstrual stages put together. The data of the HSCORES are expressed as mean ± SEM. a, $P < .005$ vs proliferative phase; b, $P < .05$ and c, $P < .005$ vs the control subjects, respectively; d, $P < .005$ vs the control subjects; e, $P < .001$ vs the control subjects as well as the eutopic endometrium of women with endometriosis; f, $P < .001$ vs the control subjects; g, $P < .01$ vs the control subjects.
ment, along with its marked decrease in the ESCs during the secretory phase when the serum P level is elevated. We also observed a significant increase in the Pak4 immunoreactivity in the eutopic endometrium of women with endometriosis and found that the Pak4 expression in the stromal cells was not different between the proliferative and the secretory phase within the endometriosis group, whereas there was a significant decrease of Pak4 expression in the secretory phase within the control subjects.

These findings suggest that P-induced down-regulation of Pak4 may be blunted in the stromal cells of eutopic endometrium of women with endometriosis, which seems to be quite similar to those of our previous report on Pak1 (16). Because we found a significant decrease of Pak4 after P treatment only in ESCs without obvious alteration in Ishikawa cells, one may argue that the effect of P can be different in normal endometrial glandular cells. However, given the in vivo immunohistochemistry results also showing obvious

Figure 4. Pak4 expression (a) and cell viability (b) after the transfection of Ishikawa cells (A) and ESCs (B) with Pak4 siRNA and nonspecific control siRNA. Data are expressed as mean ± SEM. *P < .05 vs cells transfected with nonspecific control siRNA.
Pak4 alteration only in the ESCs during the secretory phase when serum P level is high, it is more likely that our Ishikawa cell data well reflect normal endometrial glandular cells, at least in the Pak4 expression after P treatment.

Proinflammatory cytokines have been proposed to play a critical role in the integrated inflammatory cascade associated with endometriosis (25–31). However, very little is known about whether and how inflammatory cytokines can regulate Pak expression, although it has been shown that Pak4 is required for optimal binding of TNF receptor type 1-associated death domain protein to the activated TNF-α receptor and plays a role in regulating prosurvival pathways activated by TNF-α (11). As far as we know, only 1 study demonstrated that the expression of Pak1 is elevated through the stabilization of the protein after TNF-α exposure in dermal fibroblast (32). The present study showed that endometrial cells treated with TNF-α for 24 hours led to a significant increase of Pak4 expression, whereas no difference was found at 48 hours. It is not clear why the effect of TNF-α disappeared when the treat-

Figure 5. Pak4 expression (a) and cell invasion (b) after the transfection of Ishikawa cells (A) and ESCs (B) with Pak4 siRNA and nonspecific control siRNA. Data are expressed as mean ± SEM. *P < 0.05 vs cells transfected with nonspecific control siRNA.
ment time increased. Despite no data, it is possible that TNF-α up-regulates Pak4 expression just immediately after treatment and the change remains obvious within 24 hours, but not to 48 hours, which might be a characteristic of Pak4 signaling pathway. It might be also speculated that the cellular activity at 48 hours is not good enough to express Pak4 in response to TNF-α due to the unfavorable media condition extended to longer period in serum-free status. It is also unclear why IL-1β had no discernible effects on Pak4 expression in the present study. It might be suggested that IL-1β plays its role through other pathways than Pak4 activation in endometriacal cells. It is also possible that IL-1β can have its effects on Pak4 expression just immediately after treatment, which was not evaluated in the present study. The findings of the present study along with our previous study (17) suggest that proinflammatory cytokines could play a crucial role in the pathogenesis and/or pathophysiology of endometriosis by activating Pak signaling pathways and that each cytokine has a differential effect on each Pak subfamily.

It is very difficult to explain whether the increased expression of Pak4 in the eutopic endometrium and endometriotic cyst of women with endometriosis is primarily due to the possible resistance to the action of P or the secondary consequence from increased level of proinflammatory cytokines in the peritoneal environment. Given the findings of the present study that the down-regulation of Pak4 by P is observed only in the ESCs and the Pak4 expression is also higher during the proliferative phase when the effect of P is negligible in the stromal cells of the eutopic endometrium of women with endometriosis compared with the controls, it may be speculated that the Pak4 expression is affected more by the effect of TNF-α than the possible P resistance. Considering that TNF-α has been shown to inhibit the hormone-induced transcriptional activity of P receptors A and B by the activation of nuclear factor-κB (33), it is possible that increased level of TNF-α due to the presence of endometrioma reduces the expression of P receptors in endometrial cells, leading to diminished P response and failure to suppress Pak4 expression.

We could not use endometrium from absolutely disease-free controls in this study. Instead, we recruited those with leiomyoma or carcinoma in situ, assuming that their endometrium is normal. In addition, although we have shown the potential effect of Pak4 in endometriosis development using the siRNA study, the results would be much more persuasive if we had a cell model with over-expressed Pak4 because we found an increased expression of Pak4 in endometriosis in this study. Despite these limitations, the present study has shown for the first time that Pak4 is regulated by P and TNF-α and suggested that the up-regulation of Pak4 expression might lead to establishment and progression of endometriosis through enhanced cellular viability and invasiveness of endometrial cells.

Acknowledgments

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Disclosure Summary: The authors have nothing to disclose.

References


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