CXCL8 enhances proliferation and growth and reduces apoptosis in endometrial stromal cells in an autocrine manner via a CXCR1-triggered PTEN/AKT signal pathway

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Background: Chemokine CXCL8 (also known as IL-8) has been identified as a potential regulator of endometrial stromal cells (ESCs), but it is unclear how CXCL8 regulates the survival of ESCs in the pathogenesis of endometriosis.

Methods: We assessed the secretion of CXCL8 by enzyme-linked immunosorbent assays and the expression of its receptors, CXCR1 and CXCR2, by in-cell Western assay and immunohistochemistry. The effects of CXCL8 on the activation or expression of various cell mediators were also investigated by in-cell Western assay. The effects of CXCL8 on the proliferation, growth and apoptosis of ESCs in vitro were assessed by BrdU assays, cell counts and annexin V labeling, respectively.

Results: Secretion of CXCL8 and expression of CXCR1 in the eutopic ESCs from women with endometriosis were significantly higher than that in control ESCs, but the expression of CXCR2 showed no significant difference between these two cell types. CXCL8 stimulated proliferation and growth and reduced apoptosis of ESCs in an autocrine manner, and these effects were abolished by anti-human CXCL8 and CXCR1 neutralizing antibodies and by a PI3K/Akt inhibitor. Moreover, CXCL8 up-regulated the expression of the anti-apoptotic proteins, survivin and Bcl-2, inhibited the expression of the phosphatase and tensin homolog (PTEN) and activated the phosphorylation of Akt.

Conclusions: This study suggests that CXCL8 and CXCR1 are involved in the pathogenesis of endometriosis by up-regulating proliferation and growth and restricting apoptosis in ESCs by activating the PTEN/Akt pathway and mediating the expression of survivin and Bcl-2.

Key words: CXCL8 / ESCs / proliferation / apoptosis / endometriosis

Introduction

Endometriotic lesions are commonly defined by the presence of both endometrial stromal and epithelial cells, but most research focuses on the former cell type (Clement et al., 1990). The pathogenesis of endometriosis remains controversial despite extensive research. The most widely accepted theory is that the exfoliated menstrual endometrial cells attach to the peritoneal serous membrane, and their subsequent proliferation and invasion into the underlying tissue result in endometriotic lesions (Witz et al., 1999; Nisolle et al., 2000). A growing body of evidence indicates that the primary defect in endometriosis may be located in the eutopic endometrium. Abnormalities inherent to the eutopic endometrium, which are not found in the endometrium of disease-free women, might therefore contribute to the ectopic

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growth outside the uterine cavity (Ulukus et al., 2006; Szymanowski, 2007). Spontaneous apoptosis of eutopic and ectopic endometrial tissues is impaired in women with endometriosis, and this reduced susceptibility to apoptosis might permit the growth, survival and inva-
sion of endometriotic tissue (Gebel et al., 1998; Imai et al., 2000; Szy-
manowski, 2007).

Chemokine CXCL8 (also named IL-8) is the ligand of CXCR1 and
CXCR2, and is produced by many cell types, such as peripheral blood
monocytes (Yoshimura et al., 1987), mesothelial cells (Arici et al.,
1996) and endometrial cells (Arici et al., 1993). In addition to its
chemotactic (Matsushima et al., 1988), angiogenic (Koch et al.,
1992) and activating properties for granulocytes, CXCL8 is also
found to stimulate the proliferation of many other cell types (Tuschil
et al., 1992; Schadendorf et al., 1993) and to inhibit apoptosis in human breast cancer cells (Ginestier et al., 2010). Our previous
work has shown that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
can coordinate with estradiol to stimulate the production of
pro-inflammatory cytokine CXCL8 by endometriotic-associated cells
(such as endometrial stromal cell (ESC), human pelvic mesothelial
cells (HPMCs) and the human monocytic cell line U937) in vitro, and
further evoke and aggravate the inflammatory response (Shi et al.,
2006). Moreover, Arici et al. (1998) have found that CXCL8 can di-
directly promote the proliferation of EScs. However, there are still ques-
tions whether and how CXCL8 regulates the proliferation, growth and apoptosis of EScs in an autocrine dependent manner.

Therefore, the present study was undertaken to analyze the regula-
tory roles of CXCL8 in proliferation and apoptosis of EScs and to in-
vestigate the possible mechanism. Our current results illustrate that
CXCL8 can enhance the survival and growth of EScs by inhibiting
PTEN expression and activating Akt signal pathways. Hence, we
propose that the abnormal over-expression of CXCL8 and its recep-
tor CXCR1 in EScs may play an important role in the origin and de-
velopment of endometriosis.

Materials and Methods

Tissue collection, and cell isolation

All tissue samples were obtained with informed consent in accordance
with the requirements of the Research Ethics Committee in the Obstetrics
and Gynecology, Fudan University Shanghai Medical College. Samples of the
endometriotic peritoneal lesions (n = 5) and ovarian lesions (n = 5)
were obtained from women in the age of 28–45 years undergoing lapar-
oscopy for pain or other benign indications. Eutopic endometrial
tissues were obtained from fertile women (aged 22–48 years) with (n = 12) or
without (n = 18) endometriosis. None of the women had received hor-
monal medication in the 3 months prior to the surgical procedure. All
the endometrial tissues were collected under sterile conditions and
transported to the laboratory on ice in DMEM (Dulbecco’s modified
Eagle’s medium)/F-12 (Gibco, USA) supplemented with 10% fetal calf
serum (FCS; Hyclone, Logan, UT, USA). The ESCs were isolated according to
methods described previously (Li et al., 2011). The endometrial tissues
were digested with collagenase type IV (0.1%; Sigma, USA) for 30 min at
37 °C with constant agitation for recovering EScs. The tissue pieces were
filtered through sterile gauzes pads (pore diameter sizes: 200 mesh) to
remove cellular debris. Following gentle centrifugation, the supernatant
was discarded and the cells were resuspended in DMEM/F-12. The
ESCs were separated from epithelial cells by passing them over sterile
gauzes pads (pore diameter sizes: 400 mesh). The filtered suspension
was layered over Ficoll and centrifuged at 800g for 20 min to further
remove leukocytes and erythrocytes, and the middle layer was collected
and then washed with D-Hanks solution. The ESCs were placed in a
culture flask and allowed to adhere for 20 min. The adherent stromal
cells were cultured as a monolayer in flasks with DMEM/F-12 supplemen-
ted with 10% FCS and 20 mmol/l HEPES and incubated in a humidified
incubator with 5% CO2 at 37 °C. This method supplied 95% vimentin-
positive and cytokeratin-negative EScs.

Enzyme-linked immunosorbent assay for
determination of CXCL8

ESCs (2 × 10² cells/well) derived from the endometrium from women
with (n = 10) or without (n = 10) endometriosis were seeded in
24-well plates, and the supernatant was collected after 24, 48 and 72 h
in culture, respectively. The culture supernatant was harvested, centri-
fuged to remove cellular debris and then stored at −80 °C until being
assayed by ELISA. The CXCL8 concentration in the supernatant was
quantified by ELISA kits (R&D Systems, USA) according to the manufac-
turer’s instructions. Each experiment was carried out in triplicate.

In-cell Western assay

According to the description by Egorina et al. (2006) and our previous
procedure (Li et al., 2010), we used a newly set-up assay called in-cell
Western to determine the in-cell protein level of interest. Normal EScs
(n = 6) or eutopic EScs with endometriosis (n = 6) were seeded at a
density of 2 × 10⁶ cells/well in a 96-well plate for 48 h, then an in-cell
Western assay was performed to evaluate the expression of CXCR1
and CXCR2 in normal and endometriosis-associated eutopic EScs.

The procedure for subsequent studies was as follows. First 2 × 10⁶
cells/well eutopic EScs were seeded in a 96-well plate, and then the
cells were then starved for 12 h with DMEM containing 1% FBS before
treatment. After stimulation with recombinant human CXCL8 (100 ng/
ml; R&D Systems, USA) for 24 h, the cells were treated with anti-CXCL8
neutralizing antibody (R&D Systems) or with a mouse isotype control
(1 μg/ml; Sino-American Co., Ltd), or with the PI3K/Akt inhibitor,
LY294002 (50 μmol/l; Cell Signaling Technology, USA), for another
24 h. The cells were then immediately fixed with 4% formaldehyde for
20 min at room temperature. After washing with 0.01% Triton, the cells
were blocked by adding 150 μL of Li-COR Odyssey Blocking Buffer
(LI-COR Biosciences, Lincoln, Nebraska, USA) for 90 min at room tem-
perature. The cells were incubated with mouse anti-human CXCR1 or
CXCR2 (20 μg/ml, R&D Systems), mouse anti-human phospho-Erk1/2
(1:50, Santa Cruz Biotechnology, USA), rabbit anti-human Erk1/2 (1:80,
Santa Cruz Biotechnology), rabbit anti-human phospho-Akt (1:50,
Santa Cruz Biotechnology), goat anti-human Akt (1:80, Santa Cruz Biotechnol-
ogy), mouse anti-human phospho-JNK (1:50, Santa Cruz Biotechnology),
rabbit anti-human JNK (1:80, Santa Cruz Biotechnology), mouse anti-
human phospho-p38 (1:50, Santa Cruz Biotechnology), rabbit anti-human
p38 (1:80, Santa Cruz Biotechnology), rabbit anti-human PTEN or survivin
(20 μg/ml, R&D Systems) or goat anti-human Bcl-2 (20 μg/ml; Cell Sig-
aling Technology, USA) antibody, with anti-actin antibody as the internal
control. After overnight treatment at 4 °C, the wells were incubated with
the corresponding secondary antibodies: IRDye™ 700DX-conjugated af-
finity purified (red fluorescence) anti-mouse antibody and IRDye™
800DX-conjugated affinity purified (green fluorescence) anti-rabbit
antibody. However, for Akt and Bcl-2 detection groups, the wells were
incubated with the corresponding secondary antibodies: IRDye™
700DX-conjugated affinity purified (red fluorescence) anti-rabbit antibody

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and IRDye™ 800DX-conjugated affinity purified (green fluorescence) anti-goat fluorescent labeled antibody recommended by the manufacturer (Rockland, Inc., Gibertsville, PA, USA). This procedure was carried out in the dark. Images of the target genes were obtained using the Odyssey Infrared Imaging System (LI-COR Biosciences GmbH Bad, Homburg, Germany). The expression levels of the corresponding molecules were calculated as the ratio of the intensity of the target genes to actin or as the total molecules (such as total Akt or Erk1/2). The experiments were carried out in triplicate and repeated three times.

Immunohistochemistry

Paraffin sections (5 μm) of the endometrial and eutopic endometrial tissues, from women with or without endometriosis and from the proliferative or secretory phase of the cycle, were dehydrated in Tris-buffered saline (TBS) and incubated with hydrogen peroxide in 1% bovine serum albumin/TBS to block endogenous peroxidase. The samples were then incubated with mouse anti-human CXCL8 monoclonal antibody (25 μg/ml), mouse anti-human CXCR1 antibody (25 μg/ml) or mouse IgG isotype antibody overnight at 4°C in a humid chamber. After washing three times with TBS, the sections were overlaid with peroxidase-conjugated goat anti-mouse IgG antibody (SP-9002; Golden Bridge International, Inc., China), and the reaction was developed with 3,3-diaminobenzidine, and counterstained with hematoxylin. The experiments were repeated five times.

BrdU cell proliferation assay, cell number count and apoptosis assay

The BrdU cell proliferation assay and an annexin V-FITC assay were utilized to evaluate the effects of CXCL8 on cell proliferation and apoptosis, respectively, while cell growth was assessed by counting cell numbers. ESCs from women with endometriosis were resuspended in DMEM/F-12 supplemented with 10% FBS and seeded at a density of 1 × 10⁴ cells/well in 96-well flat-bottom microplates for the BrdU cell proliferation assay, or at 1 × 10⁵ cells/well in 24-well flat-bottom microplates for the apoptosis assay, or at 1 × 10⁵ cells/well in 12-well flat-bottom microplates for counting cell numbers. Thereafter, the cells were starved with DMEM containing 1% FBS for 12 h before treatment. After stimulation with recombinant human CXCL8 protein (100 ng/ml) for 24 h, the cells in wells were treated with an anti-CXCL8, CXCR1 or CXCR2 neutralizing antibodies or with LY294002 (50 μM/ml) for another 24 h. Alternatively, mouse isotype (1 μg/ml; Sino-America Co., Ltd) or vehicle was added as the negative control.

Before and after treatment, the cells in 12-well flat-bottom microplates were counted at a magnification of ×100. The results were observed under the microscope (Olympus 1X71; Olympus, Tokyo, Japan) fitted with a digital camera (Olympus DP72; Olympus). The cells were counted in five predetermined fields. Each experiment was carried out in triplicate and repeated three times.

The ability of ESCs to proliferate was assessed using the BrdU cell proliferation assay (Millipore, USA) according to the manufacturer’s instruction. Each experiment was performed in triplicate and repeated four times.

As a marker of apoptosis, phosphatidylserine externalization was quantified by flow cytometry by using a commercially available annexin V-FITC apoptosis detection kit (Invitrogen, USA) according to the manufacturer’s guideline. ESCs were trypsinized and collected. The culture medium was also retained and pooled with the adherent cells. Cells were centrifuged and the supernatant was discarded. The cells were resuspended in PBS, and washed twice and resuspended in the kit-binding buffer (100 ml/pellet) containing annexin V solution (5 μl/pellet) and propidium iodide (2.5 mg/ml). Samples were incubated in the dark for 15 min, and the percent of annexin V-positive cells in ESCs was determined by FACSCalibur flow cytometry. The experiments were performed in triplicate and repeated three times.

Statistics

All values are shown as the mean ± SD. Data were analyzed by using one-way analysis of variance and least significant difference (equal variances assumed), or Tamhane’s test (equal variances not assumed) was used post hoc for multiple comparisons by Statistical Package for the Social Sciences software version 11.5. Differences were considered as statistically significant at P < 0.05.

Results

CXCL8 and CXCR1 expression is elevated in the eutopic ESCs associated with endometriosis

The secretion of CXCL8 and the expression of CXCR1 and CXCR2 in the primary eutopic ESCs from women with or without endometriosis were evaluated by ELISA and in-cell Western assay, respectively. As depicted in Fig. 1, the secretion of CXCL8 was markedly increased in the eutopic ESCs associated with endometriosis compared with that of the normal ESCs at 48 and 72 h (P < 0.05; Fig. 1a). The expression of CXCR1 in the eutopic ESCs associated with endometriosis was higher than that in the normal ESCs (P < 0.05; Fig. 1b). However, there was no difference in CXCR2 expression between these two groups (P > 0.05; Fig. 1b).

Moreover, immunohistochemistry was used to localize CXCL8 and CXCR1 proteins in the paraffin sections. In contrast to normal endometrium, the eutopic endometrium associated with endometriosis and endometriotic tissues showed both epithelial and stromal cells that were strongly stained for CXCL8 and CXCR1 (Fig. 1c). These results suggest that higher expression of CXCL8 and CXCR1 in the eutopic ESCs derived from women with endometriosis may be associated with their unique biological characteristics.

CXCL8 induces the proliferation and growth and reduces apoptosis of ESCs in an autocrine manner

To clarify the influence of CXCL8 on ESC survival and growth, we first investigated its effect on proliferation, growth and apoptosis of ESCs. Data presented in Fig. 2 demonstrated that rhCXCL8 notably promotes proliferation (P < 0.05; Fig. 2a left) and growth (P < 0.05; Fig. 2a right), and inhibits apoptosis in eutopic ESCs (P < 0.05; Fig. 2c). Moreover, anti-CXCL8, anti-CXCR1 or the combination of anti-CXCR1 and anti-CXCR2 neutralizing antibodies decreased proliferation (P < 0.05; Fig. 2a left) and growth (P < 0.05; Fig. 2a right), and promoted apoptosis (P < 0.05 or P < 0.01; Fig. 2c) in ESCs, but anti-CXCR2 antibody alone had no effect. The increase in proliferation (P < 0.05; Fig. 2b left) and growth (P < 0.05; Fig. 2b right), and the decrease in apoptosis (P < 0.05 or P < 0.01; Fig. 2d) of the eutopic ESCs caused by CXCL8 could be significantly reversed by anti-CXCL8, anti-CXCR1 or the combination of anti-CXCR1 and anti-CXCR2 neutralizing antibodies, but not anti-CXCR2 antibody alone. Our results indicate that CXCL8 can enhance proliferation...
CXCL8 and CXCR1 expression is elevated in the eutopic ESCs with endometriosis. The secretion of CXCL8 was determined by ELISA (a), and expression of CXCR1 and CXCR2 was analyzed by in-cell Western assay (b), respectively. The relative protein levels of CXCR1 and CXCR2 in ESCs were compared between the endometriosis-associated eutopic ESCs and normal ESCs (b). The expression of CXCL8 and CXCR1 in the eutopic endometrium from women with or without endometriosis and in endometriotic tissues were analyzed, respectively, by immunohistochemistry (c). Results were highly reproducible in three independent experiments. Normal ESC is ESC from endometrium from women without endometriosis; eutopic ESC is ESC from the eutopic endometrium from women with endometriosis. Original magnification ×200. Error bars depict the standard error of the mean. \( * P < 0.05 \) compared with the normal ESC control.
Figure 2  CXCL8 induces proliferation and growth and inhibits apoptosis of ESCs in an autocrine manner. Eutopic ESCs [1 × 10⁴ cell/well, (a) left, (b) left; 1 × 10⁵ cell/well, (a) right, (b) right, (c, d)] from women with endometriosis were treated, respectively, with recombinant human CXCL8 (100 ng/ml) for 24 h, and neutralizing antibodies to CXCL8 (15 μg/ml), CXCR1 (15 μg/ml) or CXCR2 (15 μg/ml) or vehicle as controls for another 24 h. Thereafter, BrdU proliferation assays (a left, b left), cell number counts (a right, b right) and annexin V-FITC apoptosis detection assays (c, d) were used to analyze proliferation, growth and apoptosis of ESCs, respectively. Results were highly reproducible in three independent experiments. Error bars depict the standard error of the mean. *p < 0.05, **p < 0.01 compared with the vehicle control. #p < 0.05, ##p < 0.01 compared with the CXCL8 treatment.
and reduce apoptotic behavior of ESCs in an autocrine dependent manner involving the CXCR1 receptor.

CXCL8 down-regulates PTEN expression and activates phosphorylation of Akt

PI3K/Akt and MAPK signaling pathways are involved in the modulation of cell survival and growth, and CXCL8/CXCR1 can inhibit apoptosis in human breast cancer cells via activation of the FAK/Akt signal pathway (Ginestier et al., 2010). Therefore, we investigated whether CXCL8 regulates the survival and growth of the eutopic ESCs via the PI3K/Akt and/or MAPK signaling pathways. First, we detected the effect of CXCL8 on these signal pathways. As shown in Fig. 3, CXCL8 markedly stimulated the conversion of Akt to phosphor-Akt ($P < 0.01$; Fig. 3a and b) and down-regulated the expression of PTEN ($P < 0.05$; Fig. 3c), and these effects were abolished by anti-CXCL8 neutralizing antibody ($P < 0.05$ or $P < 0.01$; Fig. 3a, b, c). However, CXCL8 or anti-CXCL8 neutralizing antibody did not influence the phosphorylation levels of Erk1/2, JNK and p38 MAP kinase compared with the vehicle control ($P > 0.05$). Therefore, there exists the possibility that CXCL8 modulates ESCs survival through inhibition of PTEN expression and by activating the Akt signal pathway.

CXCL8 up-regulates survivin and Bcl-2 expression by activating the Akt signal pathway

To further analyze the possible mechanism of CXCL8 on the survival of the eutopic ESCs, we used in-cell Western assay to measure the effect of CXCL8 on the survival-related molecules in ESCs, such as PCNA (Beliard et al., 2004), survivin (Ueda et al., 2002) and Bcl-2 (Meresman et al., 2000; Beliard et al., 2004). As shown in Fig. 4, CXCL8 notably increased the expression of survivin and Bcl-2 ($P < 0.01$; Fig. 4a and b), but did not change the expression of PCNA ($P > 0.05$; Fig. 4a and b). Furthermore, these effects were completely inhibited by the pI3-kinase/Akt inhibitor, LY294002 ($P < 0.05$ or $P < 0.01$; Fig. 4a and b). As expected, the phosphorylation of Akt was also inhibited by LY294002. Our results indicate that CXCL8 may up-regulate the expression of survivin and Bcl-2 in ESCs by activating the Akt signal pathway.

The effect of CXCL8 on proliferation, growth and apoptosis of ESCs is partly mediated by the Akt signal pathway

In order to evaluate whether CXCL8 regulates survival of ESCs through the Akt signal pathway, after treatment with CXCL8 for 24 h, the eutopic ESCs from women with endometriosis were
subsequently treated with or without LY294002 for another 24 h. As shown, LY294002 not only directly inhibited proliferation, growth and increased apoptosis in the ESCs (P < 0.05 or P < 0.01; Fig. 5a left, 5a right, 5b), but also reversed the increase in proliferation and growth and decrease in apoptosis induced by CXCL8 (P < 0.05 or P < 0.01; Fig. 5a left, 5a right, 5b). Our observations suggest that the effects of CXCL8 on stimulating proliferation and growth and inhibiting apoptosis of ESCs might be mediated by the Akt signal pathway.

Discussion

Despite more than a century of intensive study, the pathophysiology of endometriosis remains unclear. A large body of evidence suggests that immune system alterations play critical roles in the development and maintenance of this enigmatic disorder, in addition to hormonal, genetic and environmental factors (Noble et al., 1996; Lebovic et al., 2001; Moutsatsou and Sekeris, 2003; Bischoff and Simpson, 2004; Dmowski and Braun, 2004; Louis et al., 2005).

IL-8 (CXCL8) is one of the pro-inflammatory chemokines secreted by several cell types, such as trophoblasts and endometrial cells and especially immune cells (Yoshimura et al., 1987). Several studies have found that human eutopic endometrium of healthy women expresses IL-8 (Shi et al., 2006; Ulukus et al., 2009), which may be involved in physiological reproductive processes as pathological processes, such as endometriosis, through regulating the proliferation and invasion of ESCs (Arici et al., 1999; Ulukus et al., 2005).

Analogous to other studies (Arici 2002; Ulukus et al., 2005), the present study has also demonstrated that the expression of IL-8 and its receptor, CXCR1, in the eutopic ESCs from women with endometriosis is higher than that of the normal ESCs from women without...
endometriosis, but the expression of CXCR2 shows no difference between these two groups. Subsequently, we have further found that recombinant human CXCL8 can enhance proliferation and growth and inhibit apoptosis of ESCs, and these effects can be inhibited by both CXCL8 and CXCR1 neutralizing antibodies. These findings indicate that the ESC-derived CXCL8 regulates the biological functions of ESCs at least partly through binding with the CXCR1 receptor. However, CXCR2 blocking has no this effect on ESCs. The underlying mechanism of this differential effect on ESC survival mediated by CXCR1 and CXCR2 is still unclear, and requires further research.

Gebel et al. (1998) found for the first time that the pattern of apoptosis in the eutopic endometrium of women with endometriosis is lower than that of the normal eutopic endometrium from healthy controls. Apoptosis of both endometrial stroma and glands in endometrium associated with endometriosis was lower compared with that in endometrium from women without endometriosis (Szymanowski, 2007). Taking into account our results, we hypothesize that in women with endometriosis an abnormally high level of CXCL8 secretion may reduce apoptosis in the eutopic and ectopic endometrial tissue, which favors ectopic growth, survival and invasion.

Interestingly, we have further demonstrated in the present work that CXCL8 regulates the expression of survival-relative molecules, survivin and Bcl-2, inhibits the expression of PTEN and modulates the proliferation, growth and apoptosis of ESCs by activating the Akt signal pathway, but not by influencing the MAPK signal pathway.

Figure 5  The effect of CXCL8 on proliferation, growth and apoptosis of ESCs is partly mediated by the Akt signal pathway. After the eutopic ESCs from women with endometriosis were treated, respectively, with recombinant human CXCL8 for 24 h, these cells were treated with or without LY294002 for another 24 h, or with vehicle as control. Thereafter, proliferation (a, left), cell number (a, right) and apoptosis (b) of ESCs were detected by BrdU proliferation assays, cell number counts or annexin V-FITC apoptosis assays, respectively. Results were highly reproducible in three independent experiments. Error bars depict the standard error of the mean. *P < 0.05, **P < 0.01 compared with the vehicle control. #P < 0.05, ##P < 0.01 compared with the CXCL8 treatment.
Survivin is an inhibitor of apoptosis expressed during fetal development and in cancer tissues. Several studies have demonstrated that survivin and Bcl-2 play an important role in physiological homeostasis during the normal menstrual cycle, and up-regulation of survivin and Bcl-2 may contribute to survival and or invasion of ESCs in the progression of endometriosis (Konno et al., 2000; Ueda et al., 2002). Therefore, our current results led us to propose that an increase in survivin and Bcl-2 expression, via the activation of the Akt signal pathway, mediated by IL-8, may enhance the survival of ESCs.

Ginestier et al. (2010) established that the effects of CXCR1 blockade on viability of breast cancer stem cells (CSCs) and FASL production were mediated by the FAK/Akt/FOXO3a pathway. In addition, as a small-molecule CXCR1 inhibitor, selectively depleting the CSC population in human breast cancer cell lines in vitro, repertaxin was able to specifically target the CSC population in human breast cancer xenografts, retarding tumor growth and metastasis. These data suggest that CXCR1 blockage may provide a novel means of targeting and eliminating breast CSCs. Endometriosis exhibits a great degree of variability not only in the symptomatic presentation, but also in terms of age of onset, progression of the disease, response to treatment and recurrence. Our present study shows that the chemokine CXCL8 may be a stimulatory factor to the survival of ESCs in the pathogenesis of endometriosis, and a CXCR1 blocker can effectively impair these roles. Therefore, CXCR1 blockage is potential for therapeutic treatment of endometriosis.

In conclusion, our findings suggest that the increase of CXCL8 and CXCR1 expression in the eutopic ESCs of women with endometriosis may promote the expression of survivin and Bcl-2, and enhance ESC survival via regulating PTEN/Akt signal pathways. Our previous work has established that estrogen and TCDD can stimulate secretion of cytokine CXCL8 in human endometrial stromal cells. Therefore, interference with cytokine regulatory loops such as CXCL8 and CXCR1 may represent a novel therapeutic strategy to reduce the growth of endometriosis.

**Authors’ roles**

M.Q.L. and X.Z.L. conducted all experiments and prepared the figures and the manuscript. Y.H.M and J.M. assisted with in-cell Western analyses. X.Y.Z. examined patients, obtained specimens and generated clinical data. L.P.J. critically reviewed and corrected the manuscript. D.J.L. initiated and supervised the project and edited the manuscript.

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**Conflict of interest**

None declared.

**References**


None declared.