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Basal and Steroid Hormone-Regulated Expression of CXCR4 in Human Endometrium and Endometriosis

Abigail Ruiz, BS[1], **Virgilio A. Salvo, MD**[2], **Lynnette A. Ruiz, BS**[1], **Perla Báez, MT**[1], **Miosotis García, MD**[3], and **Idhaliz Flores, PhD**[1]

[1]Department of Microbiology, Ponce School of Medicine, Ponce, PR, USA

[2]Department of Physiology, Pharmacology and Toxicology, Ponce School of Medicine, Ponce, PR, USA

[3]Department of Pathology, Ponce School of Medicine, Ponce, PR, USA

Abstract

Endometriosis is associated with activation of local and systemic inflammatory mechanisms, including increased levels of chemokines and other proinflammatory cytokines. We have previously reported increased gene expression of chemokine receptor 4 (*CXCR4*), the receptor for CXCL12, in lesions of the rat model of endometriosis. The CXCR4-CXCL12 axis has been shown to have both immune (HIV infection, lymphocyte chemotaxis) and nonimmune functions, including roles in tissue repair, angiogenesis, invasion, and migration. There is evidence indicating that these mechanisms are also at play in endometriosis; therefore, we hypothesized that activation of the CXCR4-CXCL12 axis could be responsible, at least in part, for the survival and establishment of endometrial cells ectopically. Immunohistochemistry (IHC) showed that CXCR4 protein levels were significantly higher in endometriotic lesions compared to the endometrium of controls. Next, we determined basal gene and protein expression of CXCR4 and CXCL12 and regulation by estradiol (E2) and/or progesterone (P4) in endometrial cell lines using quantitative polymerase chain reaction (qPCR), and Western blots. Basal *CXCR4* gene expression levels were higher in epithelial versus stromal cells; conversely, *CXCL12* was expressed at higher levels in stromal vs epithelial cells. *CXCR4* gene expression was significantly downregulated by ovarian steroid hormones in endometrial epithelial. These data suggest that steroid modulation of CXCR4 is defective in endometriosis, although the specific mechanism involved remains to be elucidated. These findings have implications for future therapeutic strategies specifically targeting the inflammatory component in endometriosis.

Keywords

endometrium; endometriosis; chemokines; CXCR4; CXCL12; ovarian steroid hormones; estradiol; progesterone; gene expression; immunohistochemistry

Corresponding Author: Idhaliz Flores, Department of Microbiology, Ponce School of Medicine, PO BOX 7004, Ponce, PR 00731, USA, iflores@psm.edu.

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Introduction

Endometriosis is a complex, estrogen-dependent, inflammatory disease defined as the presence of endometrial glands and stroma outside the uterine cavity.[1] It is a very common gynecologic diagnosis, with an estimated prevalence of up to 10% among all premenopausal women.[2] Ectopic growth of this otherwise normal tissue causes inflammation, fibrosis, adhesions, and ovarian cysts, which result in chronic pelvic pain, dyspareunia, dysmenorrhea, and infertility. The peritoneum, ovaries, cul-de-sac, and uterine ligaments are the most common areas affected, but endometriosis lesions have also been reported at extraperitoneal sites.[3] The etiology of endometriosis is unknown, but it is presumably a multifactorial disorder, resulting from aberrant expression of multiple susceptible genes, exposure to environmental factors, and dysregulated immune/inflammatory responses.[4,5] As a result, various cellular mechanisms are activated, which presumably allow survival, adhesion, proliferation, and invasion of endometrial cells that reach the peritoneal cavity by retrograde flow.[6–8] The development of endometriosis may be a result of hypo- or hyperactivation of immune factors that induce a permissive environment leading to survival of endometrial cells ectopically.[9] In fact, there is strong evidence to support the role of immune and inflammatory mechanisms in endometriosis.[9–15]

The human chemokines are small (8–12 kD) polypeptides characterized by having proinflammatory actions.[16,17] The chemokine system includes approximately 40 chemokines and 20 receptors. Chemokine receptors are 7-transmembrane domain proteins that belong to the G-coupled receptor superfamily.[18,19] Chemokines are known to promote chemotaxis and endothelial cell adhesion of leukocytes but also neoplastic and cancer cells.[16] The chemokine receptor 4 (CXCR4) and its specific ligand, CXCL12 (also know as stromal-derived factor 1 [SDF-1]), are reported to act in a paracrine fashion in cancer, promoting tumor growth and development, angiogenesis, and metastasis to tissues where *CXCL12* is expressed.[20] In addition, expression of *CXCR4* has been correlated with metastatic potential, disease severity, risk of recurrence, and unfavorable prognosis in several cancers.[21–24] At the molecular level, CXCL12 binding to CXCR4 induces expression of metalloproteinases (eg, MMP1, MMP13), connective tissue remodeling enzymes that facilitate the metastatic process in cancer.[25,26] In addition, CXCL12-CXCR4 interaction promotes angiogenesis by attracting endothelial cells to the tumor microenvironment and inducing the expression of VEGF.[27]

In regard to endometriosis, several members of the chemokine family have been implicated in the development of this disease including CCL2, CCL5, CXCL1, and CXCL8.[9,28–31] For at least 2, CCL2 and CCL5, expression was positively correlated with disease stage. In addition, *CXCL12* expression by Tregs was shown to be increased in a baboon model of endometriosis and a role for chemokines in the observed natural killer cell defect reported in endometriosis has also been proposed.[32] Using global gene expression profiling, we have previously reported an increase in *CXCR4* gene expression levels in the lesions of a rat model of endometriosis.[33] In humans, CXCR4 messenger ribonucleic acid (mRNA) has been previously shown to be upregulated in ovarian endometriosis as compared to normal ovary,[34] and in glandular epithelium of both ectopic and eutopic endometrium from patients

with endometriosis compared to eutopic endometrium from control women.[9,35] Interestingly, *CXCR4* expression levels in ovarian endometriosis were comparable to those in ovarian cancer tissues and both were higher than their normal counterpart (ie, normal ovarian tissue), and *CXCL12* gene expression in endometriosis was higher than both ovarian cancer and normal ovary.[34]

Based on these data, we hypothesized that the CXCR4-CXCL12 axis is involved in endometriosis by promoting the growth and invasion of endometrial cells at ectopic sites. The aim of the current study was, therefore, to add to the current knowledge on the role of inflammatory molecules on the pathophysiology of endometriosis by determining protein expression levels and localization of CXCR4 in human endometriotic and endometrial tissue samples. In addition, since the lesion environment is hyperestrogenic and endometriotic lesions show abnormal responses to ovarian steroid hormones, we hypothesized that dysregulations of the CXCR4-CXCL12 axis homeostasis may play a role in endometriosis. We therefore, also studied whether *CXCR4* and *CXCL12* gene expression could be modulated in vitro by ovarian steroid hormones. These studies support a role of inflammation and specifically of the CXCR4-CXCL12 axis in endometriosis and provide evidence in support of this axis as a possible target for therapeutic intervention for this puzzling disease (Table 1).

Materials and Methods

Immunohistochemistry of CXCR4 in Human Endometrium and Endometriotic Samples

Deidentified formalin-fixed paraffin-embedded (FFPE) endometrium and endometriosis tissues were obtained from a collaborating pathology laboratory after protocol approval by the Ponce School of Medicine Institutional Review Board (IRB) Committee. All biopsies were evaluated by a pathologist who confirmed diagnosis of endometriosis and stage of the menstrual cycle of eutopic samples using Noyes criteria.[36] A total of 8 endometrial and 11 endometriotic human samples were histologically evaluated and 10 μm sections were cut for immunostaining. After dewaxing and rehydration, antigen retrieval was conducted (Vector H-3300, Vector Laboratories, Burlingame, California) followed by the removal of endogenous peroxidase activity using standard methods. The endometriotic and eutopic endometrial tissue sections were incubated overnight at 4°C with primary antibody against CXCR4 (1:300 dilution; mouse immunoglobulin G[IgG]; R&D Systems, Minneapolis, Minnesota) diluted in nonimmune block solution. Sections were incubated for 30 minutes at room temperature (RT) with the secondary biotinylated antibody following the manufacturer's recommendations (Vectastain Elite ABC kit, Vector Laboratories, Inc), Signals were developed with diaminobenzidine (DAB) and counterstained with Meyer hematoxylin solution. For the negative controls, tissues were incubated with 3% normal serum (mouse IgG; Vector Laboratories). Positive controls were sections of breast cancer lymph nodes. The slides were mounted and visualized at 10, 20, and 40× magnification on an inverted microscope with an Olympus 35 mm camera (Nikon, Japan). Immunostaining intensity was evaluated in each endometrial compartment (epithelium, glands, and stroma,) using a semiquantitative method. Staining intensity was blindly scored as absent (0), weak (1), moderate (2), or intense (3) by 3 independent observers. Median immunohistochemistry

(IHC) intensity score and interquartile range were calculated per compartment per sample for statistical analyses.

Cell Culture and Ovarian Steroid Hormone Treatments

The human endometrial stromal (HESC) and epithelial endometrial cells (EEC) cell lines, kindly donated by Dr Asgerally Fazleabas (Michigan State University), were cultured as previously described.[37,38] All cells were maintained at 37°C in a humidified incubator with 5% CO_2. For the steroid hormone treatments, 1 million cells were plated per well on a 6-well plate in 10% fetal bovine serum (FBS) media for 24 hours. Media was then removed and changed to serum-deprived (ie, 1% FBS) media containing estradiol (E2; 10^{-8} mol/L), progesterone (P4; 10^{-7} mol/L), or both for 24 hours. At the end of the treatments, cells were washed and trypsinized for RNA and protein isolation. All experiments were done thrice.

Quantitative Polymerase Chain Reaction Analysis of *CXCR4* and *CXCL12* Gene Expression in Endometrial Cell Lines

Total RNA was isolated using RNAeasy kit (Qiagen, Valencia, California) following standard protocols, and quantified using a Biophotometer (Eppendorf, Hauppauge, New York). After treatment with DNAse, total RNA (1 μg) was reversetranscribed using complementary DNA (cDNA) synthesis kit according to the manufacturer's protocol (Bio-Rad, Hercules, California). The cDNAs generated were diluted 1:20 and 4.5 μL were amplified using specific TaqMan Gene Expression Assays (Applied Biosystems, Foster City, California; CXCR4: Hs00976734_m1, CXCL12: Hs00171022_m1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control for normalization purposes (Hs99999905_m1). Quantitative polymerase chain reaction (qPCR) reactions were performed in triplicate on an *i*Cycler (Bio-Rad) using the following conditions: 12.5 μL of TaqMan Universal PCR Master Mix (2X), 1.25 μL of TaqMan Gene Expression Assay Mix (20X), and the diluted cDNA. Reaction conditions were 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds and 60°C for 1 minute. Gene expression levels were calculated according to the $2^{-\Delta\Delta Ct}$ method as described by Livak et al.[39]

Western Blot Analysis of CXCR4 Protein Expression in Endometrial Cell Lines

Cells were washed 3 times in phosphate buffered saline (PBS), detached using a cell scraper and centrifuged for 10 minutes at 2000g at 4°C. The pellet was resuspended in lysis buffer containing 2% sodium dodecyl sulfate (SDS) in PBS, supplemented with proteinase and phosphatase inhibitors. Cell lysates were boiled 5 minutes, and passed 3 times through a 27-gauge needle. Total protein concentration was quantified using Bio-Rad Protein assay, and 10 μg of total proteins were electrophoresed in 10% SDS-polyacrylamide gels on a Mini-protean III system (Bio-Rad), blotted onto a nitrocellulose membrane for 1 hour at 4°C, and stained with Ponceau S (Sigma-Aldrich, St. Louis, Missouri) to verify uniformity of sample loading and transfer. Membranes were blocked with 5% skim milk in Tris-buffered saline with 0.05% Tween-20 (Tween-Tris buffered saline [TTBS]) for 2 hours, and subsequently incubated in a 1:300 dilution of the primary antibody (anti-CXCR4, R&D Systems, Minneapolis; anti-GAPDH—Santa Cruz Biotechnology, Santa Cruz, California) overnight

at 4°C. Membranes were washed 3 times with TTBS and incubated with horseradish peroxidase-conjugated secondary antibody (1:2,500 dilution) for 1 hour at room temperature. Finally, membranes were washed 3 times with TTBS and the enhanced chemiluminescence was analyzed by autoradiography (Pierce-Thermo Fisher Scientific, Rockford, Illinois).

Statistical Analyses

Quantitative polymerase chain reaction results obtained were reported as mean ± SE of at least 3 experiments in triplicate. Immunohistochemistry results were reported as median intensity score and interquartile range. Quantitative polymerase chain reaction and IHC results were analyzed by nonparametric independent sample *t* test (Mann-Whitney) or nonparametric analysis of variance (ANOVA) using SPSS 15.0 (Chicago, Illinois). Statistical significance was set at $P < .05$.

Results

Immunostaining of CXCR4 Protein in Human Endometrium and Endometriotic Tissues

The protein expression and localization of CXCR4 was studied in FFPE endometriotic lesions and endometrium from control women. Positive immunostaining for CXCR4 was detected in stromal, glands, and epithelium in both diseased and control tissues; however, the intensity was significantly higher in all cellular compartments of the endometriotic lesions when compared to eutopic endometrium of controls. In glandular epithelium, CXCR4 immunostaining was predominantly localized in the cytoplasm, toward the lumen (Figure 1). In the stromal compartment, however, immunostaining was evident in both cytoplasm and nuclei.

Basal *CXCR4* and *CXCL12* Gene Expression in Human Endometrial Cell Lines

We then determined the basal level of expression of the *CXCR4* and *CXCL12* genes in human endometrial cell lines. CXCR4 and CXCL12 mRNA expression was evaluated in stromal (HESC) and EEC by qPCR. We observed that *CXCR4* gene expression was significantly higher in epithelial than in stromal endometrial cells (61-fold higher; Figure 2A). CXCL12, in contrast, was highly expressed (117-fold higher) by endometrial stromal cells but was barely detectable by qPCR in endometrial epithelial cells (Figure 2B). Western blot analysis confirmed the qPCR results for CXCR4 (Figure 2C).

Ovarian Steroid Hormone Regulation of *CXCR4* and *CXCL12* Gene and Protein Expression in Human Endometrial Cell Lines

Next, we determined the transcriptional regulation of *CXCR4* and *CXCL12* genes by E2 and/or P4 in vitro. Gene expression of *CXCR4* was significantly downregulated by ovarian hormone treatments in endometrial epithelial (E2: 0.4-fold; P4: 0.4-fold; E2+P4: 0.3-fold; $P = .0081$), but not in stromal cells (Figure 3A). *CXCL12* gene expression, however, was downre-gulated by steroid hormone treatments in endometrial stromal cells (E2: 0.4-fold; P4: 0.3-fold; E2+P4: 0.3-fold; $P = .0053$), but not in epithelial cells (Figure 3B). Using Western blots at the basal level (control vehicle or CV), 2 specific bands were detected with the anti-CXCR4 antibody (50 and 47 kD) (Figure 4). All treatments with ovarian steroid

hormones caused the loss of the higher molecular weight band in epithelial cells. However, no significant downregulation in the CXCR4 protein levels correlating with the mRNA findings were observed. In stromal cells, however, there were no apparent differences in *CXCR4* expression at the protein level. This observation is in accord with the qPCR results showing that changes in *CXCR4* expression at the mRNA level also did not reach statistical significance.

Discussion

Chemokines are known to be potent proinflammatory mediators and more recently they have been ascribed roles in cancer, as regulators of angiogenesis, tissue remodeling, invasion and metastases, cell growth, and proliferation.[17] Based on our observation that endometriotic lesions in a rat model of endometriosis express higher levels of the chemokine receptor CXCR4 than normal endometrium,[33] that *CXCR4* gene expression is increased in both ectopic and eutopic endometrium of patients with endometriosis,[9,34,35] and that the peritoneal fluid of patients contains high levels of chemokines,[9,29–31] we hypothesized that the CXCR4-CXCL12 axis is a key player in the pathophysiology of this disease by promoting invasion and growth of endometrial cells ectopically. We specifically aimed to help clarify the role of this axis in endometriosis by determining levels of expression in human tissues and also assessing *CXCR4* and *CXCL12* gene expression regulation by ovarian steroid hormones in normal endometrial cell lines.

To assess the pattern of *CXCR4* expression in vivo, we conducted IHC on endometriosis biopsies and ectopic endometrium from control women. We observed a significantly higher expression of *CXCR4* in stroma and epithelium from endometriotic tissues as compared to secretory endometrium. These data are highly relevant since endometriotic lesions, despite their heterogeneity (different menstrual cycle phase, localization) on average expressed higher levels of *CXCR4* than endometrium from controls, all of which were in the secretory phase, when the expression of this chemokine receptor is maximal.[40] Our observation that endometriotic tissues express high levels of CXCR4 protein is in accord with 2 studies that analyzed cell-specific gene expression in endometriosis lesions or endometrial biopsies from endometriosis patients by cDNA microarrays.[9,35] Using laser capture microdissection (LCM) isolated epithelial cells, both studies showed that glandular epithelium expressed higher levels of CXC chemokine receptors, including CXCR4, than eutopic endometrium or normal ovary from controls, respectively. Moreover, *CXCR4* and *CXCL12* have been previously shown to be expressed at higher levels in epithelium of ovarian endometriosis and ovarian cancer tissue when compared to normal ovary.[34] In fact, ovarian endometriosis has been proposed as a risk factor for ovarian cancer, although ectopically growing endometriotic cells transform at very low rates.[41–43] Interestingly, we observed nuclear staining of CXCR4 predominantly in the stromal component of the endometriotic lesions. Nuclear localization of CXCR4 and activation of metastatic signaling cascades on binding to its ligand have been previously reported.[44] Taken together, these results suggest that the CXCR4-CXCL12 axis plays a role in both endometriosis and ovarian cancer and adds on to the growing evidence in support of shared pathophysiological mechanisms in these disorders.

Since endometriotic lesions are characterized by high endogenous E2 levels, and show abnormal responses to ovarian steroid hormones, we hypothesized that dysregulations of the CXCR4-CXCL12 axis homeostasis resulting from aberrant hormonal responses may explain, at least in part, the phenotype of endometriotic cells.[45,46] Others have shown that chemokines (eg, CXCL8, CCL2, and CCL5) are hormonally regulated in human endometrium, and that *CXCR4* and *CXCL12* expression in vivo is variable during the menstrual cycle.[47,48] Therefore, we then analyzed the basal and steroid hormone regulated expression of *CXCR4* and *CXCL12* in normal endometrial cells. In vitro, we observed that *CXCL12* was highly expressed by stromal endometrial cells (HESC) and barely undetectable in epithelial cells, as expected. In contrast, basal *CXCR4* expression was more pronounced in epithelial than stromal cells. We observed that ovarian steroid hormones (P4 and E2+P4) significantly downregulated *CXCR4* gene expression levels in normal endometrial epithelial cells, and that *CXCL12* gene expression was also significantly downregulated by E2+P4 in endometrial stromal cells. The significant changes in *CXCR4* gene expression observed in EEC are not comparable to its protein levels, which could be explained in various ways, including posttranscriptional (eg, mRNA stability) and posttranslational modifications (eg, protein degradation), which may affect the half-life of mRNA and/or protein. That mRNA and protein levels are not always comparable has been well described in the literature, indicating the importance of measuring gene expression at both mRNA and protein levels as done in the current study.[49,50] Interestingly, steroid hormone treatment appeared to regulate *CXCR4* expression at the post-translational level in epithelial cells, shown by the loss of 1 of the 2 CXCR4-specific bands observed. *CXCR4* has been previously reported to be modified by glycosylation and sulfation.[51,52]

These data are in disagreement with previously reported results by Dominguez and colleagues, showing that in vivo the expression of *CXCR4* slightly increased in luminal epithelium and endothelial cells during the window of implantation (WOI) compared to the proliferative phase.[53] Based on this observation we expected to see an increase in the expression of *CXCR4* following E2+P4 treatment, corresponding to the higher level of expression seen in humans during WOI. These conflicting results may be explained by the fact that *CXCR4* expression is modulated by a variety of factors (eg, CXCL12, interferon γ [IFNγ], transforming growth factor β [TGFβ], hypoxia, epigenetic regulation, HER2 coexpression),[54,55] and also may be due to the short incubation time (24 hours) used in these studies.[56,57] These results could also be explained by the fact that in vivo stromal and epithelial interact and possibly regulate paracrine hormonal responses, which cannot be replicated using cell lines grown individually in vitro. More studies are evidently necessary to elucidate the mechanisms whereby *CXCR4* gene expression is regulated in vivo in both normal and pathologic endometrium.

Our data show that ovarian steroid hormones, and P4 in particular, modulate *CXCR4* and *CXCL12* gene expression, generally maintaining low levels of these molecules in epithelial and stromal cells, respectively. In endometriotic tissues, it seems, such regulation is lost, as CXCR4 protein expression was significantly elevated in the endometriotic lesions in all subcellular components (ie, glands and luminal epithelial, as well as stromal cells) compared to eutopic secretory endometrium of control women. The well-characterized defects in the

expression of estrogen receptor α (ER-α) and progesterone receptor (PR) in endometriosis may lead to the loss of CXCR4 regulation by ovarian steroid hormones, its increased and sustained expression, and activation via local high levels of its stromal cell-derived ligand.[58,59] Since *CXCR4* has been reported as having cancer-promoting functions (ie, induction of invasion, metastasis, angiogenesis, cell proliferation), these findings have important implications for better understanding the pathophysiology of endometriosis, which indeed shares these phenomena with cancer.[41–43] However, it remains to be determined whether chemokines produce the same effects in cancer and endometriosis.[60]

The high levels of *CXCR4* seen in the endometriotic lesions could result from a single or a combination of factors. Regardless of the mechanisms of CXCR4 upregulation, binding of CXCL12 to its specific receptor in endometriotic cells could result in the activation of signal transduction mechanisms that may contribute to (1) increased metalloproteinase gene expression—key molecules involved in connective tissue remodeling and, therefore, in cancer cell migration and metastasis, and (2) sustained growth of endometriotic cells and neovascularization of the lesion thereby facilitating survival. Which signal transduction pathway is activated will lead to a different cell fate: increased growth and proliferation without motility (re-epithelialization—characterized by matrix metalloproteinase 1 [MMP-1] expression) versus motility and invasion (chronic wound repair—characterized by MMP-13 expression).[61] More studies on which signal transduction mechanisms are activated in the endometriotic lesions via the CXCL12-CXCR4 interactions (eg, actin polymerization, Erk, p38MAPK, c-Jun, and/or GSK3α/β signaling), and the functionality of this axis in context of endometrial cells are warranted.[19,21,62] These studies will help better understand why endometriotic cells share molecular mechanisms with cancer cells but do not usually transform and rarely metastasize.[47]

In summary, the current study showed that protein expression of CXCR4 is increased in endometriotic tissues and pointed at possible mechanisms mediating this observation. These findings imply that the CXCR4-CXCL12 axis may play an important role in the genesis and maintenance of endometriotic lesions and that the observed increased expression of the CXCR4 receptor may be explained by aberrant responses to ovarian steroid hormones. However, due to the role of the CXCR4-CXCL12 axis in diverse cellular processes that are relevant to this disease (eg, tissue regeneration, cell motility, invasion, apoptosis, angiogenesis) more studies are necessary to elucidate the nature of the specific involvement of this system in endometriosis. Finally, since CXCR4 antagonists have been shown to have the potential as antitumor drug,[63–65] we propose that the small peptide inhibitors of CXCR4 could also become a therapeutic option for endometriosis.

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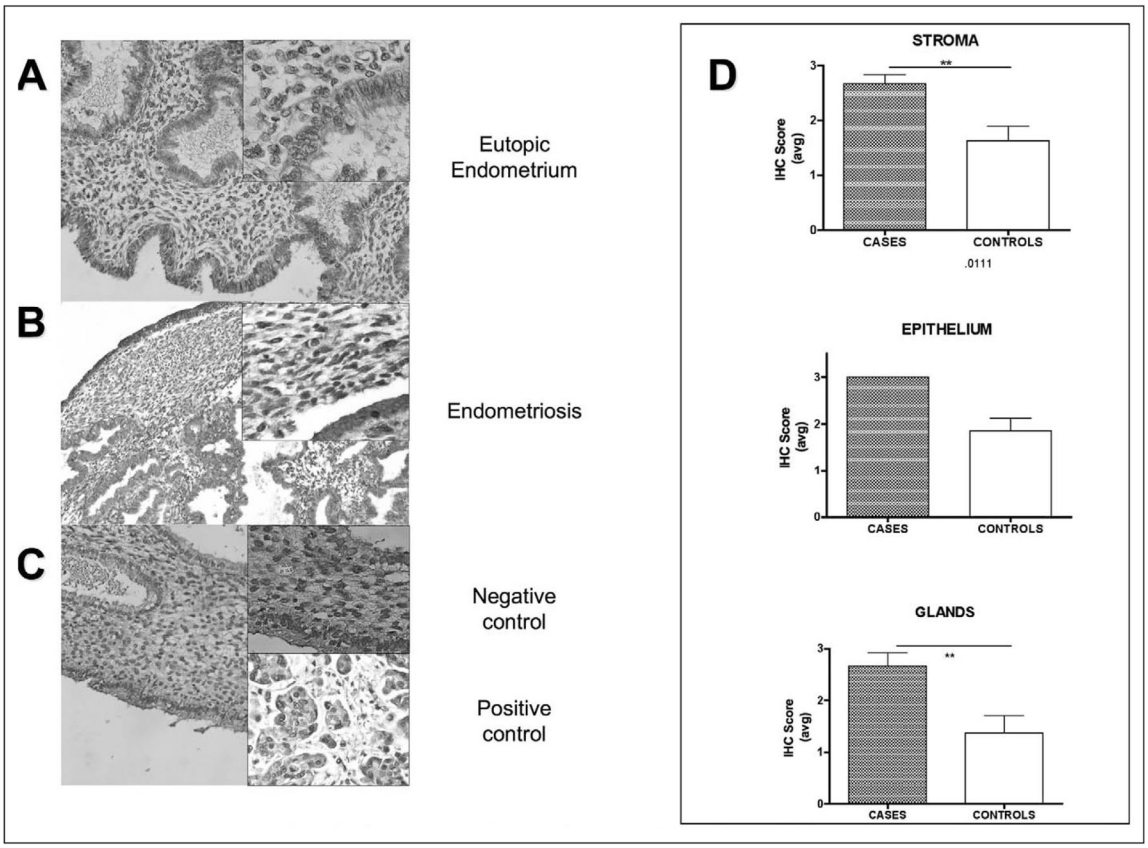

Figure 1.

Immunohistochemical localization of CXCR4 protein in ectopic and eutopic human endometrium. A, A representative normal endometrium sample (n = 8) immunostained for *CXCR4*. B, Endometriosis lesions (n = 11) stained for *CXCR4*. C, Endometrial section with 3% normal serum (mouse immunoglobulin G [IgG]) antibody (negative control) and breast cancer-infiltrated lymph node as positive control. Representative pictures at ×10 magnification (insets at ×20) are shown. Immunohistochemistry staining score for CXCR4 in human endometrial and endometriosis tissue. The staining intensity was evaluated and scored by 3 independent observers using the intensity scale (3 = strong, 2 = moderate, 1 = weak, 0 = no staining). An asterisk (*) represents significant difference between groups.

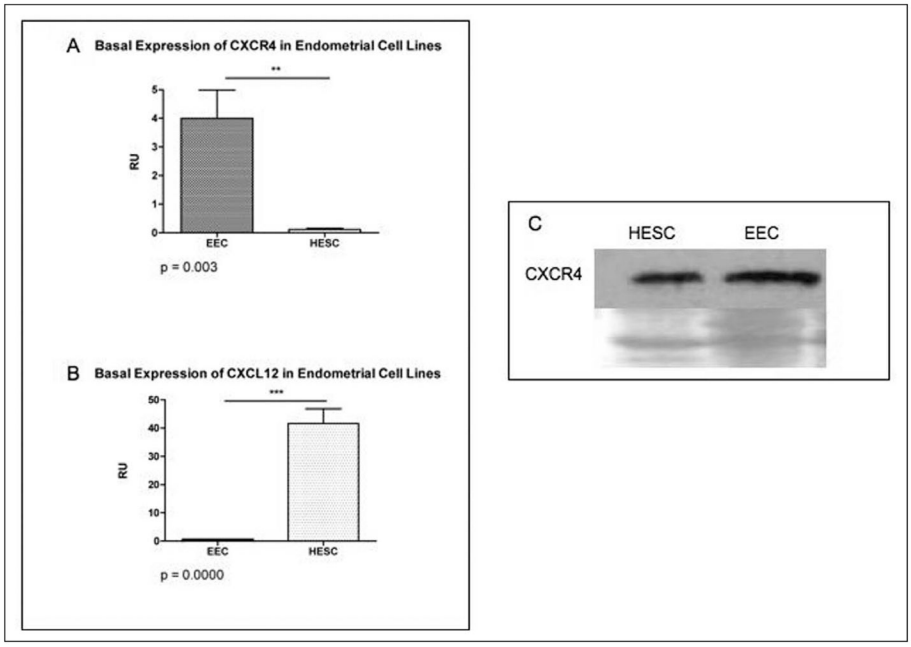

Figure 2.

Basal expression of *CXCR4* and *CXCL12* in human endometrial cell lines. *CXCR4* (A) and *CXL12* (B) gene expression was determined by quantitative polymerase chain reaction (qPCR) 24 hours after 90% confluent growth in complete media. Data from 3 experiments in triplicate is presented as box and whisker plots. Representative Western blot analysis of CXCR4 protein expression (C). Cells were cultured in complete media and total protein was isolated. CXCR4 protein was detected by immunostaining. Ponceau staining was used as loading control.

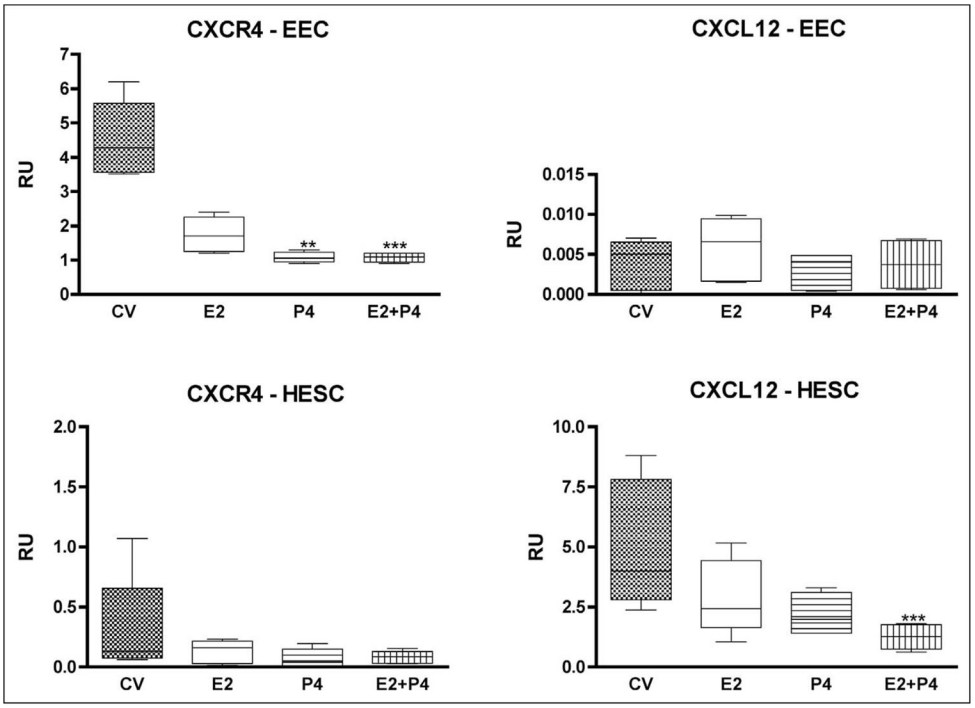

Figure 3.

Ovarian steroid hormone regulation of *CXCR4* and *CXCL12* gene expression in human endometrial cell lines. *CXCR4* and *CXCL12* gene expression was determined by quantitative polymerase chain reaction (qPCR) after 24 hours treatment with estradiol (E2), progesterone (P4), and the combination of both (E2+P4). Data from 3 experiments in triplicate is presented as box and whisker plots.

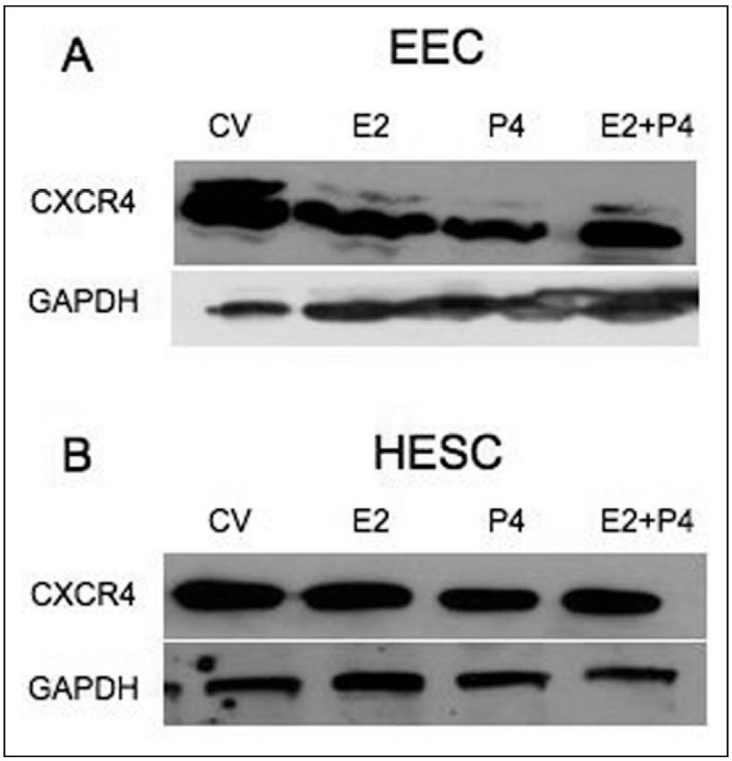

Figure 4.

Ovarian steroid hormone regulation of CXCR4 protein expression in human endometrial cell lines. Representative Western blot analysis of CXCR4 protein expression. Epithelial cells (EEC) (A) and stromal (HESC) (B) cells were treated with 10^{-8}mol/L of estradiol (E2) and/or 10^{-7}mol/L progesterone (P4) for 24 hours. Controls were cells treated with medium plus vehicle (CV). Total protein was isolated and expression was detected by immunostaining. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control for normalization purposes.

Table 1

Demographic Characteristics of Patients With Endometriosis and Controls[a]

ID#	Age	Endometrial Dating	Diagnosis
120	48	Secretory	Metrorrhagia
105	52	Secretory	Menometrorrhagia
196	44	Secretory	AUB
438	20	Secretory	
686	30	Secretory	
700	41	Secretory	
1246	37	Secretory	Fibroids
4036	37	Secretory	
Average age controls: 38.6 years old (range 20–52)			
4151	30	Proliferative	Cul-de-sac endometriosis
2732	36	Proliferative	Endometrioma, fibroids
12270	32	Unknown	Endometrioma
78575	47	Proliferative	Fallopian tube endometriosis; leiomyoma
239	35	Proliferative	Leiomyomata uteri; fallopian tube endometriosis
1824	44	Secretory	Ovarian and fallopian tube endometriosis; leiomyoma
2726	40	Secretory	Ovarian endometriosis; adenomyosis
2390		Secretory	CPP; endo stage II
1254	28	Secretory	Endometriosis
18885	48	Proliferative	Fallopian tube endometriosis
15565	25	Secretory	Cervix endometriosis; endo stage III; CPP; AUB

Abbreviations: AUB, abnormal uterine bleeding; CPP, chronic pelvic pain.

[a] Average age cases: 36.5 years old (range: 25–48).