Role of Estrogen Receptor-β in Endometriosis

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Endometriosis is an estrogen-dependent disease. The biologically active estrogen, estradiol, aggravates the pathological processes (e.g., inflammation and growth) and the symptoms (e.g., pain) associated with endometriosis. Abundant quantities of estradiol are available for endometriotic tissue via several mechanisms including local aromatase expression. The question remains, then, what mediates estradiol action. Because estrogen receptor (ER)β levels in endometriosis are >100 times higher than those in endometrial tissue, this review focuses on this nuclear receptor. Deficient methylation of the ERβ promoter results in pathological overexpression of ERβ in endometriotic stromal cells. High levels of ERβ suppress ERα expression. A severely high ERβ-to-ERα ratio in endometriotic stromal cells is associated with suppressed progesterone receptor and increased cyclo-oxygenase-2 levels contributing to progesterone resistance and inflammation. ERβ-selective estradiol antagonists may serve as novel therapeutics of endometriosis in the future.
pain management. Patients often develop resistance to repeated treatments with the same agent over a period of 6 months to 3 years. Because an average patient may need repeated treatments with various drugs during her reproductive lifespan, there is a clear need to identify novel molecular pathways that can be targeted with emerging therapeutic agents.

Here we discuss the mechanisms responsible for the strikingly high expression of estrogen receptor (ER)β and its targets in endometriosis. This low response rate is likely further reduced with repeated treatment attempts. New translational perspectives include treating endometriosis with new ERβ-selective compounds. Some consideration has been given to determining the effect of an ERβ ligand on endometriosis. No further clinical information, however, has come forth. ERβ-selective compounds may constitute a new class of drugs for the treatment of endometriosis-associated pain in the future.

**Gene Expression in Endometrium and Endometriosis**

We use the terms *endometriotic tissue* or *endometriosis* interchangeably in reference to the endometrium-like tissues that are present in the pelvic peritoneum or ovaries. However, the terms *endometrial tissue* or *endometrium* refer to uterine mucosa, which is appropriately located within the uterine cavity. A biological distinction is also made between endometrium from disease-free women versus women with endometriosis. Sampson proposed the most widely accepted mechanism for the development of endometriosis on pelvic peritoneal surfaces as the implantation of endometrial tissue on the peritoneum through retrograde menstruation. Because retrograde menstruation occurs in >90% of all women, endometriosis is believed to be caused by molecular defects that favor survival and establishment of endometrial tissue in menstrual debris on the peritoneum. Gene expression profiles characterized by microarray in the endometrium of women with or without endometriosis showed that a large number of genes were dysregulated. These findings suggested that the eutopic endometrium of women with endometriosis exhibit the pathology found within endometriotic tissue. This abnormal pattern of gene expression could also be traced to primary stromal cells isolated from endometriotic tissue, eutopic endometrium from endometriosis, and eutopic endometrium from disease-free women.

**Estrogen Production in Endometriosis**

Estradiol and progesterone are master regulators of endometrial tissue. Each steroid hormone is estimated to regulate expression of hundreds of genes during various phases of the menstrual cycle. Endometriotic and eutopic endometrial tissues respond to estradiol and progesterone with apparently similar histological changes, and both tissues contain immunoreactive estrogen and progesterone receptors (PRs). The eutopic endometrium predictably becomes atrophic in response to prolonged progestin therapy or oral contraceptives that contain progestins. Treatment with these agents, however, does not predictably suppress endometriotic tissue growth. Endometriotic tissue in ectopic locations, such as the peritoneum or ovary, is fundamentally different from eutopic endometrium within the uterus in terms of the production of cytokines and prostaglandins, estrogen biosynthesis and metabolism, and clinical response to progestins. There are substantial molecular differences with regard to progesterone response between normal endometrium and eutopic and ectopic tissues from women with endometriosis.

Estradiol is the biologically active estrogen. It is produced in primarily three body sites in a woman with endometriosis. In all of these sites, expression of the enzyme aromatase is essential for estradiol production; additionally, several other steroidogenic proteins are expressed and complement aromatase activity for the production of estradiol. The classical site for estrogen production is the ovary. The theca and granulosa cells of a preovulatory follicle convert cholesterol to estradiol that is actively secreted into the circulation in a cyclic fashion. The second group of body sites is collectively referred to as the peripheral tissues, including bulky tissues such as fat, skin, and skeletal muscle, all of which express aromatase. In these peripheral tissues, circulating androstenedione is converted to estrone, which is further converted to estradiol. Peripheral tissues do not secrete estradiol in a classical sense, but because of their large quantity, they produce sufficient levels of estradiol to raise its blood levels, particularly in obese women. The third site for estradiol production is the endometriotic tissue itself. The endometriotic stromal cell uniquely expresses...
the full complement of genes in the steroidogenic cascade, which is sufficient to convert cholesterol to estradiol.26

Nuclear Receptor Expression in Endometriosis

Estrogen or progesterone action is mediated primarily by their nuclear receptors, abbreviated as ER and PR. The steroids estradiol and progesterone interact with their respective receptors and activate them to act as transcription factors. In contrast to ER and PR, another group of nuclear receptors, named orphan nuclear receptors, do not have any known ligands. Steroidogenic factor (SF)1 belongs to this latter group. Because of the roles of estrogen and progesterone in endometrium, we assessed the expression of these key nuclear receptors in endometriosis (Fig. 2).

Circumstantial and laboratory evidence strongly support the notion that estradiol is a key hormone for the growth and persistence of endometriotic tissue as well as inflammation and pain associated with it. Estradiol, which reaches endometriosis by circulation or is produced locally in endometriotic tissue, acts as a steroid hormone to regulate growth of endometriotic tissue. Estradiol enters cells and binds to the ER in estrogen-responsive cells. ER subtypes α and β are proteins with high affinity for estradiol and are encoded by separate genes. The classical human ERα was cloned in 1986, and a second estrogen receptor, ERβ, was cloned from rat prostate and human testis in 1996.27–29 Although both ERα and ERβ are present in the endometrium, ERα seems to be the primary mediator of the estrogenic action in this tissue.30

Despite its sensitivity to estrogen, endometriosis appears to contain a unique complement of steroid hormone receptors compared with that of its normal tissue counterpart, the eutopic endometrium. For example, several investigators reported markedly higher levels of ERβ and lower levels of ERα in human endometriotic tissues and primary stromal cells compared with eutopic endometrial tissues and cells.31,32 The levels of both isoforms of PR, particularly PR-B, are significantly lower in endometriosis compared with eutopic endometrium.6,33

The estradiol-receptor complex acts as a transcription factor that becomes associated with the promoters of estradiol-responsive genes via direct DNA binding or binding to other docking transcription factors at basal promoter regions.34 This interaction brings about ER-specific initiation of gene transcription, which promotes the synthesis of specific mRNAs and proteins.34 PR is one of many estradiol-responsive genes, and estradiol acts in eutopic endometrial tissues and stromal cells to promote endometrial responsiveness to progesterone.35 In contrast, PR mRNA and protein levels are not elevated in biopsied endometriotic tissues exposed to high estradiol levels during late proliferative phase or in endometriotic cells treated with estradiol, indicating that estradiol-induction PR expression in endometriosis is markedly blunted.33

In addition to ERα, ERβ, and PR, the orphan nuclear receptor SF1 is also differentially regulated in endometriosis versus eutopic endometrium (Fig. 2). SF1 is responsible for coordinately activating the full steroidogenic cascade of genes including aromatase. The protein products of this set of steroidogenic genes are capable of converting cholesterol to estradiol locally in endometriotic tissue.36 We recently used real-time polymerase chain reaction (RT-PCR) to compare tissue mRNA levels of these key nuclear receptors in

![Figure 2](image-url)
endometriosis and eutopic endometrium (Fig. 2). Ovarian endometriotic tissue SF1 and ERβ mRNA levels were >12,000 times and 142 times higher than in endometrium, respectively. In contrast, ERα, PR, and PR-B levels were remarkably lower in endometriotic tissue (Fig. 2). The role of SF-1 in endometriosis has been reviewed elsewhere. This review covers primarily ERβ expression and action in endometriosis.

**Primary Endometriotic Stromal Cells Mimic Tissue Levels of ERα, ERβ, and PR mRNA and Protein Levels**

Differential expression of nuclear receptors in endometriotic versus endometrial tissues are preserved in stromal cells cultured from these tissues. Differential expression of steroid receptors in endometrial and endometriotic tissues and cells has been consistently reported. RT-PCR was used to quantify the mRNA levels of nuclear receptors in primary endometrial and endometriotic stromal cells. ERα mRNA levels were significantly lower (sevenfold) in endometriotic stromal cells compared with endometrial stromal cells. ERβ mRNA was strikingly higher (~34-fold) in endometriotic stromal cells, whereas it was much lower or nearly absent in endometrial stromal cells. The ratios of ERα to ERβ mRNA levels were, on average, 841 and 21 in endometrial and endometriotic stromal cells, respectively. Total PR and PR-B mRNA levels in endometriotic stromal cells were significantly lower than those in endometrial stromal cells.

Protein levels of ERα and ERβ were significantly different in these two groups similar to the findings regarding mRNA levels. In conclusion, ERα, ERβ and PR levels were markedly different in endometrial versus endometriotic tissues or stromal cells derived from these tissues. Endometriotic stromal cells contained extraordinarily higher ERβ and significantly lower ERα and PR levels compared with endometrial stromal cells.

**Epigenetic Regulation of the ERβ Promoter is Responsible for Its High Levels in Endometriosis**

Because of the extreme differential expression of ERβ between endometriotic and endometrial cells, we tested the hypothesis that alteration in DNA methylation is a mechanism responsible for severely increased ERβ mRNA levels in endometriotic cells. We identified a CpG island occupying the promoter region of the ERβ gene (Fig. 3). Bisulfite sequencing of this region showed significantly higher methylation in primary endometrial cells versus endometriotic cells. Treatment with a demethylating agent significantly increased ERβ mRNA levels in endometrial cells. The critical region that confers promoter activity also bears the identical CpG island (Fig. 3). The activity of the ERβ promoter was strongly inactivated by in vitro methylation. Therefore, methylation of a CpG island at the ERβ promoter region is a primary mechanism responsible for differential expression of ERβ in endometriosis and endometrium. Thus high ERβ mRNA and protein expression in endometriotic cells were mediated by an epigenetic defect involving hypomethylation of a CpG island occupying its promoter (Fig. 3).

**ERβ Is Responsible for Low ERα Expression in Endometriotic Stromal Cells**

ERα mRNA and protein levels are several fold lower in endometriotic tissue and stromal cells compared with endometrial tissue and stromal cells. ERα deficiency in endometriosis may be responsible for the failure of estradiol to induce PR expression, thus contributing to secondary PR deficiency and progesterone resistance in women with this disease. In vivo observations strongly suggest that estradiol induces ERα expression in mouse uterine tissue. It is quite likely that estradiol also plays a key role in regulating ERα expression in human endometrial stromal cells. However, strikingly high quantities of estradiol produced via local aromatase activity in addition to high ERβ levels in stromal cells of endometriosis may perturb this regulation and may suppress ERα expression.

We tested the hypothesis whether ERβ is responsible for suppressing ERα promoter activity and mRNA and protein expression in endometriotic cells (Fig. 3). The human ERα gene is regulated via multiple promoters; the three major promoters are A, B, and C and are alternatively used in various tissues. Promoters A and B are located within the 2-kb region proximal to the translation start site, whereas promoter C lies some 101 kb upstream of this site.

Primary endometriotic stromal cells in culture were utilized to determine the role of ERβ in estradiol-dependent regulation of the ERα gene. ERβ knockdown significantly increased ERα mRNA and protein levels in endometriotic stromal cells. Conversely, ERβ overexpression in endometrial stromal
cells decreased ERα mRNA and protein levels. ERβ knock- 
down significantly decreased proliferation of endometriotic stromal cells. ERβ knock- 
down or overexpression most 

We screened the three ERα promoter regions using serial 
chromatin immunoprecipitation assays for binding of ERβ or 
ERα itself. Estradiol enhanced binding of both ERα and ERβ to 
a region containing a nonclassical activator protein (AP)-1 motif in promoter A in endometriotic cells. We investigated 
several regions of ERα promoter C, which lies some 101 kb 
upstream of the common splice junction. In the presence of 
estradiol, ERβ bound to a genomic region flanking an AP1 site 
upstream of promoter C and a region bearing a specificity 
protein (Sp)1 motif immediately downstream of promoter C 
in endometriotic stromal cells. In contrast, ERα bound to 
neither promoter C sequences. In summary, these findings 
collectively suggest that primarily ERα promoter C may 
mediate ERβ-mediated inhibition of ERα expression in endo-
metriotic stromal cells (►Fig. 3). A role of ERα promoter A 
could not be ruled out. Knockdown studies also suggested a 
positive role of ERβ on endometriotic stromal cell cycle 
progression and proliferation.

Possible PR Suppression in the Presence of 
High ERβ-to-ERα Ratio in Endometriosis

ERα mediates estradiol induction of PR in malignant breast 
epithelial cells. Thus PR has been viewed as a classical ERα 
target gene. It has been reported that two distinct estradiol-
regulated promoters generate transcripts encoding the two 
functionally different human PR isoforms, PR-A and PR-B. 
Previous studies have demonstrated that maximal PR mRNA 
and protein levels are reached after human breast cancer cells 
have been exposed to estradiol for 3 days. 

Two major transcriptional start sites have been identified. The upstream 
transcription start site gives rise to a full-length mRNA 
species that encodes the PR-B protein. Another mRNA species 
with a further downstream transcription initiation site gives 
rise to the truncated PR-A form. Despite the fact that both 
proposed PR promoter sequences are estradiol responsive, 
neither contains a classical palindromic estrogen response 
element sequence. Several nonclassical regulatory ele-
ments (e.g., AP-1, Sp1) in the human PR promoter have 
been reported. These sites have been shown to bind ERα 
and on one occasion ERβ. Intriguingly, more recent 
research suggests that distal regions of the PR gene, as far as 311 
kb upstream of the PR-B transcription start site, participate in the 
dynamic regulation of this gene and that the coordinated 
action of proximal and distal PR gene regions allows cells to 
respond to changes in estradiol levels.

Our unpublished observations suggest that a reduction in the 
ERα-to-ERβ ratio in endometrial stromal cells diminishes 
estradiol induction of PR expression. Thus we speculate that a 
critical level of ERα may be necessary for estradiol-dependent 
induction of PR in endometriotic stromal cells. The occupancy of 
the PR promoter regions with varying ratios of ERα to ERβ 
may be critical in determining the effect of estradiol on PR 
expression. A profoundly low ERα-to-ERβ ratio in endometri-
otic stromal cells may be responsible for a shift from estradiol 
stimulation to failure of induction of PR expression in endo-
metriotic stromal cells (►Fig. 3).

Is ERβ Responsible for High Cox2 Expression in 
Endometriotic Stromal Cells?

ERβ is the only estrogen receptor subtype expressed in 
primary placental villus endothelial cells. In these cells, 
ERβ is essential for maintaining cyclo-oxygenase (COX)2 
mRNA and protein levels. In vitro, this effect of ERβ 
seems to be independent of the availability of estradiol. 
Because COX2 expression is also severely higher in endo-
metriotic versus endometrial tissues and cells, it is tempting 
to speculate that ERβ is at least in part responsible for high 
COX2 levels and prostaglandin biosynthesis in endometriosis (►Fig. 3).

Summary

High estrogen production is a consistently observed endo-
ctrine feature of endometriosis. Expression of steroid recep-
tors and other nuclear receptors are strikingly different 
between endometriotic and eutopic endometrial tissues. 
Among these nuclear receptors, ERβ expression is maybe 
100 times higher in endometriotic tissue than in endome-
 trium. Defective DNA methylation and other accompanying 
epigenetic mechanisms may be responsible for strikingly high 
ERβ expression in endometriosis. ERβ suppresses ERα 
expression and results in strikingly high ERβ-to-ERα ratios 
in endometriotic cells. We speculate that a strikingly lower 
ERα-to-ERβ ratio in endometriotic stromal cells may cause a shift 
from estradiol stimulation to inhibition of PR expression in 
endometriotic stromal cells under in vivo circumstances (►Fig. 3). This proposed mechanism may explain severely 
deficient PR-B in endometriotic stromal cells, which contrib-
utes to progesterone resistance in women with endometri-
osis. ERβ overexpression in endometriosis possibly has other 
broad effects important in the pathology of endometriosis. It 
is likely that ERβ simulates prostaglandin production in 
endometriotic tissues and cells via inducing COX2 expression. Thus ERβ represents a key therapeutic target for endometri-
osis-associated pain. ERβ-selective compounds that antago-
nize estradiol in endometriotic stromal cells may be the 
future therapeutics of endometriosis.

Acknowledgments

This manuscript was supported by a grant from the NICHD 
(R37-HD37691, to SEB).

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