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Estrogen Receptor- β , Estrogen Receptor- α , and Progesterone Resistance in Endometriosis

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Abstract

Loss of progesterone signaling in the endometrium may be a causal factor in the development of endometriosis, and progesterone resistance is commonly observed in women with this disease. In endometriotic stromal cells, the levels of progesterone receptor (PR), particularly the PR-B isoform, are significantly decreased, leading to a loss of paracrine signaling. PR deficiency likely underlies the development of progesterone resistance in women with endometriosis who no longer respond to progestin therapy. Here we review the complex epigenetic and transcriptional mechanisms leading to PR deficiency. The initial event may involve deficient methylation of the estrogen receptor (ER) β promoter resulting in pathologic overexpression of ER β in endometriotic stromal cells. We speculate that alterations in the relative levels of ER β and ER α in endometrial tissue dictate E2-regulated PR expression, such that a decreased ER α --ER β ratio may result in suppression of PR. In this review, we propose a molecular model that may be responsible for changes in ER β and ER α leading to PR loss and progesterone resistance in endometriosis.

Keywords

ER- β ; ER- α ; PR; progesterone resistance; DNA methylation; epigenetic; promoter; gene regulation; transcription

Endometriosis is defined as the presence of endometrium-like tissue outside of the uterine cavity. It is one of the most common causes of infertility and chronic pelvic pain and affects 1 in 10 women of reproductive age.^{1,2} The incidence of endometriosis is estimated to be as high as 30% in patients with infertility and 45% in patients with chronic pelvic pain.² Similar to other common chronic diseases, such as diabetes mellitus and asthma, endometriosis is inherited in a polygenic manner and has a complex and multifactorial etiology.³ There is a sevenfold increase in the incidence of endometriosis in relatives of women with this disease compared with those without.⁴ The most striking aspect of endometriosis is its dependence on estrogen for growth, similar to that seen in eutopic endometrium, but a lack of consistent response to progesterone or synthetic progestins.⁵

Endometriosis-related infertility is treated with assisted reproductive technologies such as in vitro fertilization. Endometriosis-related pain is primarily treated with endocrine agents such as synthetic progestins, oral contraceptives, or gonadotropin releasing hormone analogues.¹ These treatments interrupt ovulation, however, and are successful in only half of patients treated.⁶⁻⁸ Moreover, 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.

In this article, we focus on the mechanisms underlying the development of resistance to progesterone action in endometriosis. There is a clear need for a better understanding of this process because only half of patients with endometriosis-related pelvic pain respond to treatment with continuous treatment with progestins (Depo-Provera) or combination oral contraceptives.⁶⁻⁸ This low response rate is likely further reduced with repeated treatment attempts. New translational perspectives include treating endometriosis with new selective progesterone receptor modulators (SPRMs) that likely exhibit altered binding properties to progesterone receptors (PR) or target certain PR isoforms.

Another clinically useful question denotes the mechanism responsible for increased expression of estrogen receptor (ER) β in endometriosis, which in turn may affect the levels of other key nuclear receptors such as ER α and PR. Some consideration has been given to determining the effect of an ER β ligand on endometriosis.⁹ Further molecular and clinical studies may reveal the mechanism by which ER β -selective ligands may treat endometriosis-associated symptoms.

TERMINOLOGY

In this review, the terms *endometriotic tissue* and *endometriosis* refer to the pathological state in which ectopic endometrium-like tissues grow within the pelvic peritoneum or ovaries. The terms *endometrial tissue* and *endometrium* refer to normal eutopic or intrauterine endometrial tissue. 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.¹⁰⁻¹² Gene expression profiles characterized by microarray in the endometrium of women with or without endometriosis showed that several progesterone target genes were dysregulated during the window of implantation, at which time endometrium is exposed to the highest levels of progesterone.¹³ For example, the prototype progesterone-responsive gene, glycodelin, was downregulated strikingly in endometrium of women with endometriosis compared with women without

endometriosis. These findings suggested that the eutopic endometrium of women with endometriosis exhibit the pathology found within endometriotic tissue.^{13,14}

ROLES OF ESTRADIOL AND PROGESTERONE IN ENDOMETRIAL PHYSIOLOGY AND PATHOLOGY

In a postmenopausal women undergoing in vitro fertilization with a donor egg, the exogenous administration of only estradiol (E2) and progesterone is sufficient to prepare the endometrium for implantation in the absence of ovarian function. This observation underscores the essential roles of these steroids in uterine physiology. Indeed, both E2 and progesterone are master regulators of endometrial tissue. Each hormone is estimated to regulate expression of hundreds of genes during various phases of the menstrual cycle.¹⁵ Endometriotic and eutopic endometrial tissues respond to E2 and progesterone with apparently similar histological changes, and both tissues contain immunoreactive ERs and 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 production of cytokines and prostaglandins, estrogen biosynthesis and metabolism, and clinical response to progestins.^{8,16,17} There are substantial molecular differences with regard to progesterone response between normal endometrium and eutopic and ectopic tissues from women with endometriosis.^{13,18,19}

ESTROGEN ACTION IN ENDOMETRIUM AND ENDOMETRIOSIS

Both circumstantial and laboratory evidence strongly support the notion that estrogen is an extremely strong mitogen for endometriotic tissue. The biologically active estrogen E2, which is secreted by the ovary or locally produced by endometriotic tissue, acts as a classical steroid hormone to regulate growth of endometrial or endometriotic tissue. E2 enters cells and binds to the ER in estrogen-responsive cells. ER subtypes α and β are proteins with high affinity for E2 and are encoded by separate genes. The classical human ER α was cloned in 1986, and a second estrogen receptor, ER β , was cloned from rat prostate and human testis in 1996.^{20–22} Although both ER α and ER β are present in the endometrium, ER α seems to be the primary mediator of the estrogenic action in this tissue.²³

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 ER β and lower levels of ER α in human endometriotic tissues and primary stromal cells compared with eutopic endometrial tissues and cells.^{24,25} Moreover, the levels of both isoforms of PR, particularly PR-B, are significantly lower in endometriosis compared with eutopic endometrium.^{5,26}

The E2-receptor complex acts as a transcription factor that becomes associated with the promoters of E2-responsive genes via direct DNA binding or binding to other docking transcription factors at basal promoter regions.²⁷ This interaction brings about ER-specific initiation of gene transcription, which promotes the synthesis of specific mRNAs and proteins.²⁷ PR is one of many E2-responsive genes, and E2 acts in eutopic endometrial tissues and stromal cells to promote endometrial responsiveness to progesterone.²⁸ In contrast, PR mRNA and protein levels are not elevated in biopsied endometriotic tissues exposed to high E2 levels during late proliferative phase or in endometriotic cells treated with E2, indicating that E2-induction PR expression in endometriosis is markedly blunted.²⁶

ER α , ER β , AND PR mRNA AND PROTEIN LEVELS ARE DIFFERENTIALLY REGULATED IN PRIMARY ENDOMETRIOTIC VERSUS ENDOMETRIAL STROMAL CELLS

Because the response of endometriotic tissue to sex steroids is markedly different from that of endometrium, we and others determined the expression of steroid receptors in cells from these tissues. Our recent findings regarding the levels of steroid receptors in endometrial and endometriotic cells are consistent with previously published data.^{24,26} Real-time polymerase chain reaction was used to quantify the mRNA levels of nuclear receptors in primary endometrial and endometriotic stromal cells. ER α mRNA levels were significantly lower (sevenfold; Fig. 1) in endometriotic stromal cells compared with endometrial stromal cells. ER β mRNA was strikingly higher (~34-fold; Fig. 1) in endometriotic stromal cells, whereas it was much lower or nearly absent in endometrial stromal cells.²⁹ The ratios of ER α to ER β were, on average, 841 and 21 in endometrial and endometriotic stromal cells, respectively.²⁹ Total PR and PR-B mRNA levels in endometriotic stromal cells were significantly lower than those in endometrial stromal cells.²⁹ Protein levels of ER α and ER β were significantly different in these two groups similar to the findings regarding mRNA levels.²⁹ In conclusion, ER α , ER β , and PR levels were markedly different in endometrium versus endometriosis-derived stromal cells. Endometriotic stromal cells contained extraordinarily higher ER β and significantly lower ER α and PR levels compared with endometrial stromal cells.

DECREASED METHYLATION OF A CpG ISLAND AT ER β PROMOTER IS RESPONSIBLE FOR HIGH ER β LEVELS IN ENDOMETRIOSIS

Because of the extreme differential expression of ER β between endometriotic and endometrial cells, we tested the hypothesis that alteration in DNA methylation is a mechanism responsible for severely increased ER β mRNA levels in endometriotic cells.²⁹ We identified a CpG island occupying the promoter region of the ER β gene. Bisulfite sequencing of this region showed significantly higher methylation in primary endometrial cells versus endometriotic cells. Treatment with a demethylating agent significantly increased ER β mRNA levels in endometrial cells. The critical region that confers promoter activity also bears the CpG island.²⁹ The activity of the ER β promoter was strongly inactivated by *in vitro* methylation. Therefore, methylation of a CpG island at the ER β promoter region is a primary mechanism responsible for differential expression of ER β in endometriosis and endometrium.²⁹ Therefore, high ER β mRNA and protein expression in endometriotic cells were mediated by an epigenetic defect involving hypomethylation of a CpG island occupying its promoter (Fig. 2).

ER β SUPPRESSES ER α LEVELS IN ENDOMETRIOTIC STROMAL CELLS

ER α deficiency in endometriosis may be responsible for failure of E2 to induce PR expression, thus contributing to secondary PR deficiency and progesterone resistance in women with this disease. *In vivo* observations strongly suggest that E2 induces ER α expression in mouse uterine tissue.³⁰ It is quite likely that E2 also plays a key role in regulating ER α expression in human endometrial stromal cells. However, strikingly high quantities of E2 produced via local aromatase activity in addition to high ER β levels in stromal cells of endometriosis may perturb this regulation and may suppress ER α expression.^{29,31} We tested the hypothesis whether ER β is responsible for suppressing ER α promoter activity and mRNA and protein expression in endometriotic cells. The human ER α gene is regulated via multiple promoters; the three major promoters are A, B, and C and are used alternatively in various tissues.^{32–34} 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.^{32,35}

We determined the role of ER β in E2-dependent regulation of the ER α gene in endometrial and endometriotic stromal cells.³⁶ ER β knockdown significantly increased ER α mRNA and protein levels in endometriotic stromal cells.³⁶ Conversely, ER β overexpression in endometrial stromal cells decreased ER α mRNA and protein levels. ER β knockdown significantly decreased proliferation of endometriotic stromal cells.³⁶ ER β knockdown or overexpression primarily regulated ER α mRNA species arising from the far distal promoter C.

We screened the three ER α promoter regions using serial chromatin immunoprecipitation assays for binding of ER β or ER α itself (Fig. 3). E2 enhanced binding of both ER α and ER β to a region containing a nonclassic activator protein 1 (AP1) motif in promoter A in endometriotic cells (Fig. 3). We investigated several regions of ER α promoter C, which lies some 101 kb upstream of the common splice junction. In the presence of E2, ER β bound to a genomic region flanking an AP1 site upstream of promoter C and a region bearing a specificity protein 1 (Sp1) motif immediately downstream of promoter C in endometriotic stromal cells. In contrast, ER α bound to neither promoter C sequences. Taken together, these findings suggest that ER β -mediated inhibition of ER α expression may be mediated primarily by promoters A and C.

In summary, high levels of ER β suppress ER α expression and response to estradiol in endometriotic stromal cells via binding to nonclassic DNA motifs in alternatively used ER α promoters. ER β also regulates cell cycle progression and might contribute to proliferation of endometriotic stromal cells.

SEVERELY INCREASED ER β -to-ER α RATIO MAY BE RESPONSIBLE FOR DECREASED PROGESTERON RECEPTOR EXPRESSION IN ENDOMETRIOSIS

PR is a prototype target gene of ER α in several cell types including breast malignant epithelial cells. ER α mediates E2 induction of PR. It has been reported that two distinct E2-regulated promoters generate transcripts encoding the two functionally different human PR isoforms, PR-A and PR-B.³⁷ Previous studies have demonstrated that maximal PR mRNA and protein levels are reached after human breast cancer cells have been exposed to E2 for 3 days.^{38–40} 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 E2 responsive, neither contains a classical palindromic ERE sequence.⁴¹ Several nonclassic regulatory elements (e.g., AP1, Sp1) in the human PR promoter have been reported. These sites have been shown to bind ER α and on one occasion ER β .^{28,41–49}

We speculate that a critical level of ER α may be necessary for E2-dependent induction of PR in endometrial stromal cells. The occupancy of the PR promoter regions with varying ratios of ER α -to-ER β may be critical in determining the effect of E2 on PR expression. A strikingly lower ER α -to-ER β ratio in endometriotic stromal cells may be responsible for a shift from E2 stimulation to E2 inhibition of PR expression in endometriotic stromal cells (Fig. 4).

SUMMARY

Complex mechanisms involving promoter regulation may be responsible for the observed aberrations in ER α , ER β , and PR expression in endometriosis. The stromal cell component of endometriotic tissue may be the primary site of these abnormalities. In endometriotic stromal cells, ER β promoter is pathologically hypomethylated and therefore hyperactive, leading to very high ER β levels. ER β suppresses ER α expression and results in strikingly high ER β -to-ER α ratios in endometriotic cells. We speculate that a strikingly lower ER α -to-ER β ratio in endometriotic stromal cells may cause a shift from E2 stimulation to E2 inhibition of PR expression in endometriotic stromal cells under in vivo circumstances (Fig. 4). This hypothetical mechanism may be the answer for a critically important clinical question regarding the etiology of severely deficient PR-B in endometriotic stromal cells, which contributes to progesterone resistance in women with endometriosis. Lack of PR-B in the endometriotic stroma perturbs normal epithelial-stromal interactions, leading to epithelial cell defects, 17 β -hydroxysteroid dehydrogenase-2 deficiency, and progesterone resistance in endometriosis. It is possible that this consequence of PR-B deficiency is only the tip of the iceberg with regard to pathogenesis of endometriosis, and that numerous other molecular aberrations may also contribute to the development of resistance to hormone treatments in women with endometriosis. Nevertheless, an understanding of the molecular mechanisms underlying PR-B deficiency may lead to the development of new treatments that target defective pathways. This concept supports the use of SPRMs with mixed agonist/antagonist properties and possibly higher affinity to PR to overcome PR deficiency in endometriotic stromal cells. Moreover, selective ER β ligands that target high levels of ER β in endometriotic tissue may be clinically beneficial via disrupting this mechanism.

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Figure 1.

Messenger RNA levels of steroids in primary stromal cells isolated from endometrium and endometriosis ($n = 8$ patients in each category). Comparable *in vivo* differences were also observed between whole tissues of endometrium and endometriosis (our unpublished data). ER, estrogen receptor; PR, progesterone receptor; PCR, polymerase chain reaction.

Figure 2.

In endometrial stromal cells, a dense CpG island at estrogen receptor (ER) β promoter, which is heavily methylated, suppresses its expression. We speculate that this methylated CpG island is occupied by a repressor complex containing methyl-CpG binding protein-2 (MeCP2) and a corepressor (Co-Repr). In contrast, the same CpG island at ER β promoter in endometriotic stromal cells is hypomethylated permitting recruitment of an enhancer transcriptional complex including a coactivator (Co-Act).

Figure 3.

Chromatin immunoprecipitation (ChIP) polymerase chain reaction characterized estrogen receptor (ER)β or ERα binding to specific ERα promoter regions in stromal cells from ovarian endometriomas. ERα gene structure with selected potential cis-regulatory elements within each promoter region were characterized in the ChIP assay. In the presence or absence of estradiol (E2), ERβ binds via specificity protein 1 (Sp1) and activator protein (AP1) sites in ERα promoter C and the AP1 site in promoter A. In an E2-dependent manner, ERα interacts only with the ERα promoter A via an AP1 site. IgG, immunoglobulin G; PR A, promoter A; PR B, promoter B; PR C, promoter C.

Figure 4.

The proposed model for regulation of steroid receptor expression in endometriotic stromal cells. ER, estrogen receptor; PR, progesterone receptor.