Endometriosis and nuclear receptors

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BACKGROUND: Endometriosis is recognized as a steroid-dependent disorder; however, the precise roles of nuclear receptors (NRs) in steroid responsiveness and other signaling pathways are not well understood.

OBJECTIVE AND RATIONALE: Over the past several years, a number of paradigm-shifting breakthroughs have occurred in the area of NRs in endometriosis. We review and clarify new information regarding the mechanisms responsible for: (i) excessive estrogen biosynthesis, (ii) estrogen-dependent inflammation, (iii) defective differentiation due to progesterone resistance and (iv) enhanced survival due to deficient retinoid production and action in endometriosis. We emphasize the roles of the relevant NRs critical for these pathological processes in endometriosis.

SEARCH METHODS: We conducted a comprehensive search using PubMed for human, animal and cellular studies published until 2018 in the following areas: endometriosis; the steroid and orphan NRs, estrogen receptors alpha (ESR1) and beta (ESR2), progesterone receptor (PGR), steroidogenic factor-1 (NR5A1) and chicken ovalbumin upstream promoter-transcription factor II (NR2F2); and retinoids.

OUTCOMES: Four distinct abnormalities in the intracavitary endometrium and extra-uterine endometriotic tissue underlie endometriosis progression: dysregulated differentiation of endometrial mesenchymal cells, abnormal epigenetic marks, inflammation activated by excess estrogen and the development of progesterone resistance. Endometriotic stromal cells compose the bulk of the lesions and demonstrate widespread epigenetic abnormalities. Endometriotic stromal cells also display a wide range of abnormal NR expression. The orphan NRs NR5A1 and NR2F2 compete to regulate steroid-synthesizing genes in endometriotic stromal cells; NR5A1 dominance gives rise to...
Introduction

The traditional definition of endometriosis, i.e., the presence of endometrial tissue outside of the endometrial cavity, narrowly focuses on the anatomical disease, yet lesions may not always be visualized with laparoscopy, and the extent and severity of the pelvic disease may not correlate with its often debilitating symptoms, including chronic pelvic pain and infertility (Stovall et al., 1997; Milingos et al., 2003). In view of recent clinical and molecular advances in our understanding of pelvic endometriosis, it is more appropriately defined as an estrogen-dependent and inflammatory disorder associated with excessive estrogen formation. Endometriotic stromal cells show an abnormally low ESR1:ESR2 ratio due to excessive levels of ESR2, which mediates an estrogen-driven inflammatory process and prostaglandin formation. These cells are also deficient in PGR, leading to progesterone resistance and defective retinoid synthesis. The pattern of NR expression, involving low ESR1 and PGR and high ESR2, is reminiscent of uterine leiomyoma stem cells. This led us to speculate that endometriotic stromal cells may display stem cell characteristics found in other uterine tissues. The biologic consequences of these abnormalities in endometriotic tissue include intense inflammation, defective differentiation and enhanced survival.

**WIDER IMPLICATIONS:** Steroid- and other NR-related abnormalities exert genome-wide biologic effects via interaction with defective epigenetic programming and enhance inflammation in endometriotic stromal cells. New synthetic ligands, targeting PGR, retinoic acid receptors and ESR2, may offer novel treatment options.

**Key words:** endometriosis / nuclear receptors / stem cells / SF-1 / ESR2 / NR2F2 / PGR / progesterone resistance / retinoids

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![Figure 1](https://academic.oup.com/humupd/advance-article-abstract/doi/10.1093/humupd/dmz005/5365598) Examples of nuclear receptor action in endometriotic and normal uterine cells. (A) NRSA1 is an orphan nuclear receptor that directly binds to DNA as a monomer. It assembles a transcriptional complex capable of initiating the expression of steroidogenic genes in endometriotic stromal cells (Yang et al., 2002; Bulun et al., 2005). (B) In leiomyoma smooth muscle cells, a homodimer of PGRs liganded with progesterone (P) bind to a specific progesterone response element (PRE) and assemble a specific complex including a co-activator to start transcription of progesterone target genes such as BCL2 (Bulun, 2013). BCL2, B-cell lymphoma-2; C/EBPα, CREB-1, cAMP response element binding protein-α; CREB-1, cAMP response element binding protein I; CBP, CREB-binding protein; PGR, progesterone receptor; NRSA1, steroidogenic factor-1; TFs, transcription factors.
and endometriosis (Pavone et al., 2011). The NRs without known ligands, i.e. orphan NRs, steroidogenic factor-1 (NR5A1) and chicken ovalbumin upstream promoter-transcription factor II (NR2F2) also play critical roles in regulating local estrogen concentrations in endometriosis (Zeitoun et al., 1999; Attar et al., 2009; Bernardi et al., 2018).

**Methods**

A comprehensive search of the PubMed database was conducted to identify peer-reviewed literature published in English until June 2018, pertinent to the roles of NRs in endometriosis pathogenesis and progression. The search included the keywords either alone or in combination with ‘endometriosis’: estrogen receptor alpha, estrogen receptor beta, retinoic acid receptor (RAR), PGR, steroidogenic factor-1, chicken ovalbumin upstream promoter-transcription factor II, endometrial stem cells. Both animal and human studies were reviewed for this article.

**NR5A1 and NR2F2**

NR5A1, also known as SF-1, is an orphan NR because it does not have a well-defined natural ligand (Luo et al., 1994). NR5A1 plays a critical role in regulating steroidogenic pathways in the adrenal gland, testis and ovary (Luo et al., 1994; Hanley et al., 2000). While NR5A1 expression is barely detectable in the eutopic endometrium, its mRNA level is ~12,000-fold higher in endometriosis (Xue et al., 2007b). In the endometriotic stromal cells, NR5A1 is essential for supporting high levels of proteins and enzymes that drive the serial conversion of cholesterol to estradiol (Attar et al., 2009; Bernardi et al., 2018). NR5A1 binds the promoters and stimulates the expression of steroidogenic acute response protein (StAR), side chain cleavage enzyme (CYP11A1), 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2), 17β-hydroxylase/17,20-lyase (CYP17A1) and aromatase (CYP19A1) (Attar et al., 2009; Bernardi et al., 2018).

NR5A1 serves as a key downstream mediator of prostaglandin E2 (PGE2) action (Sirianni et al., 2009). Estradiol- and cytokine-induced stimulation of cyclooxygenase-2 (COX-2) leads to overproduction of PGE2, which in turn binds to its receptor on steroidogenic stromal cells and elevates intracellular levels of cAMP (Noble et al., 1997; Tamura et al., 2002, 2004; Sun et al., 2003; Wu et al., 2005). Downstream of the PGE2-cAMP pathway, NR5A1 binds to the promoters of multiple steroidogenic genes such as StAR and aromatase, which leads to formation of large quantities of estradiol (Bulun et al., 2005; Attar et al., 2009).

PGE2-induced expression of aromatase is opposed by various transcriptional repressors in the healthy endometrium, such as NR2F2 (also known as COUP-TFII) (Zeitoun et al., 1999). NR2F2 is another orphan NR that regulates a subset of genes involved in cell adhesion, angiogenesis and inflammation; these genes are also important for endometriosis progression (Li et al., 2013). Silencing Nr2f2 in cell lineages of mouse uterus results in decidualisation failure (Kurihara et al., 2007), whereas the depletion of NR2F2 in the human endometrial stromal cells selectively upregulates genes involved in inflammation and cell adhesion (Li et al., 2013). In contrast to high levels of NR5A1, NR2F2 mRNA and protein levels are significantly lower in ectopic endometriotic lesions compared with normal eutopic endometrium (Li et al., 2013; Lin et al., 2014). This downregulation may be due to the inflammatory cytokines and interleukins in peritoneal fluid in women with endometriosis, as IL-1, TNF-α and TGF-β1 have been shown to reduce NR2F2 transcript and protein levels (Lin et al., 2014). In the normal endometrium, the promoter regions of the aromatase and StAR genes are occupied by NR2F2 and not by NR5A1. Promoter-bound NR2F2 inhibits expression of steroidogenic genes, while endometriotic stromal cells override this inhibition via NR5A1 binding to chromatin with higher affinity (Zeitoun et al., 1999).

Thus, when NR5A1 is absent, NR2F2 carries out its inhibitory action on the steroidogenic pathway at various steps simultaneously to create a fail-safe system for the maintenance of normal endometrial stromal cell function in healthy endometrium (Bulun, 2009). When substantial levels of NR5A1 are present, however, NR5A1 binds to steroidogenic gene promoters with higher affinity, thus successfully competing with NR2F2 in endometriotic cells to activate the steroidogenic cascade, resulting in an estrogen-producing cell phenotype similar to that of ovarian granulosa cells.

**Regulation of NR5A1 expression and activity in endometriotic cells**

The remarkably higher expression of NR5A1 in the endometriotic stromal cells compared with normal endometrium is maintained via various epigenetic mechanisms such as DNA methylation, histone modification and post-transcriptional regulation (Bulun, 2009). Genome-wide methylation studies show that functionally important genes in endometriosis are differentially expressed in accordance with their aberrant methylation status (Dyson et al., 2014; Yotova et al., 2017). Hypomethylation of a critical CpG island at the promoter region of NR5A1 is accompanied by remarkably high NR5A1 mRNA expression in stromal cells collected from ovarian endometriomas (Xue et al., 2007b; Yamagata et al., 2014). In contrast, hypermethylation of two specific intragenic regions of the NR5A1 gene in endometriotic stroma compared to normal endometrium also increase NR5A1 expression: one CpG island spans from exon II to intron III and another is located in intron I (Xue et al., 2011, 2014). Correlation between the location of CpG islands and NR5A1 expression demonstrates that methylation of different genomic regions generates opposite effects on transcriptional activity; expression of NR5A1 increases with hypermethylation of intragenic CpG islands and hypomethylation of CpG islands around the promoter site (Dyson et al., 2014). Additionally, treating normal endometrial stromal cells with the demethylating agent 5-aza-2′-deoxycytidine induces a 55-fold increase in NR5A1 mRNA expression, reinforcing the crucial role of methylation status in determining NR5A1 expression (Xue et al., 2007b). In addition to the differential methylation status, enrichment of acetylated histones 3 and 4 in the NR5A1 promoter in endometriotic tissues shows that differential acetylation of histones may also contribute to NR5A1 overexpression (Monteiro et al., 2014). These epigenetic aberrations alter chromatin availability, allowing various transcription factors such as upstream stimulatory factor-2 to interact with the promoter region of NR5A1 in endometriotic stromal cells (Xue et al., 2007b; Utsunomiya et al., 2008).
Post-transcriptional modifications may also have a role in regulating NRSAI levels. Micro RNAs (miR) interact with mRNAs to accelerate their degradation or interfere with their translation (Bartel, 2009). Several types of miR have altered gene expression in the eutopic endometrium of women with endometriosis compared to disease-free women (Pan and Chegini, 2008; Ohlsson Teague et al., 2009; Filigheidu et al., 2010). miR23a and miR23b levels are lower in endometriosis and in the eutopic endometrium of women with endometriosis, and they inversely correlate with NRSAI levels, yet NRSAI is not a direct target of miR23a and miR23b (Shen et al., 2013). As an example of a post-translational regulatory mechanism, direct phosphorylation of NRSAI, via selective stimulation of the membrane-bound estrogen receptor GRPR30, increases NRSAI transcriptional activity, leading to induction of aromatase expression in endometriotic stromal cells (Lin et al., 2009).

**Estrogen receptor beta**

The biologically active estrogen estradiol acts via nuclear estrogen receptors to regulate growth of the endometrial tissue (Huhtinen et al., 2012). The nuclear estrogen receptor subtypes are ESR1 and ESR2, which are encoded by different genes but have 96% similarity in their DNA binding domains (Mosselman et al., 1996). While ESR1 and ESR2 are both present in the endometrium, ESR1 expression dominates, and estrogen-dependent proliferation is mediated mainly through ESR1 (Matsuzaki et al., 2001; Shang, 2006). Upon ligand binding, the estradiol/ESR1 complex acts as a transcription factor and binds to the promoters of estrogen-target genes such as the PGR to induce their expression (Weiha et al., 2000; Carroll et al., 2005; Lin et al., 2007).

Several groups have reported significantly lower levels of ESR1 both in endometriomas and cultured endometriotic stromal cells obtained from cyst walls of ovarian disease (Brandenberger et al., 1999; Fujimoto et al., 1999; Smuc et al., 2007). ESR1 mRNA expression is ~7-fold lower in stromal cells from ovarian endometriomas compared to normal endometrial stromal cells (Xue et al., 2007a). In various cancer types, methylation of CpG islands in the promoter region of the ESR1 gene is associated with gene silencing (Issa et al., 1994; Ottaviano et al., 1994). Reduced expression of ESR1 is correlated with hypermethylation of the ESR1 gene near its 3’ promoter in endometriotic stromal cells (Dyson et al., 2014). Additionally, ESR2 directly downregulates ESR1 in stromal cells derived from ovarian endometriosis (Trukhacheva et al., 2009). ESR2 binds to alternatively used promoter regions of the ESR1 in the presence of estradiol and suppresses its expression (Granden, 1996; Donaghue et al., 1999; Trukhacheva et al., 2009).

In endometriotic tissue, there is a switch from ESR1 to ESR2 dominance. RNA expression of ESR2 is 40- to 140-fold higher in stromal cells obtained from ovarian endometriomas compared to healthy eutopic endometrial stromal cells (Smuc et al., 2007; Xue et al., 2007a; Yang et al., 2015). ESR2 expression is also higher in the eutopic endometrium of women with endometriosis compared to disease-free women; suggesting that high levels of ESR2 in the endometrium increase the risk of developing endometriosis (Han et al., 2012; Monsivais et al., 2014). The abnormally high ESR2:ESR1 mRNA ratio in endometriotic stromal cells reaches an ~800-fold difference compared with normal endometrial stromal cells (Xue et al., 2007a).

The ESR2:ESR1 protein ratio is also higher in endometriotic versus endometrial stromal cells (Xue et al., 2007a).

**Cause of high ESR2 expression in endometriotic cells**

Studies investigating the association between ESR2 polymorphisms and endometriosis risk have reported inconsistent results; a meta-analysis comparing eight studies concluded that the previously suggested polymorphisms were associated with bias rather a real risk for endometriosis (Guo et al., 2014). Hypomethylation of a CpG island in the promoter of the ESR2 gene was seen in endometriotic stromal cells, whereas the same sequence was hypermethylated and therefore silenced in normal endometrial stromal cells (Xue et al., 2007a). Treatment of endometrial stromal cells with the demethylating agent, 5-aza-2-deoxycytidine, significantly increased ESR2 mRNA levels, suggesting that differential methylation is a major mechanism driving ESR2 upregulation in endometriosis (Xue et al., 2007a). DNA methyltransferase (DNMT) 1 and DNMT3B mRNA and protein are differentially expressed in endometriotic cells or lesions in comparison to normal endometrium from disease-free women (van Kaam et al., 2011; Dyson et al., 2015; Hsiao et al., 2015). Differential binding activity of DNMT3B to NRSAI and ESR1 promoters in endometriotic versus normal endometrial stromal cells suggests that this enzyme might play a role in abnormal gene expression in endometriosis (Dyson et al., 2015). The relationship between DNMT expression levels and ESR2 promoter hypomethylation in endometriosis remains to be discovered.

**Biological consequences of ESR2 overexpression in endometriosis**

Aberrantly high ESR2 levels regulate several pathological processes in endometriotic tissue including proliferation, inhibition of apoptosis, inflammation and pain transmission (Monsivais et al., 2014; Han et al., 2015). Genome-wide chromatin immunoprecipitation approaches identified a total of 70 genes that had an ESR2 binding site and that also were differentially expressed in normal endometrium versus endometriosis (Monsivais et al., 2014; Han et al., 2015). In particular, estradiol induces ESR2 enrichment at the promoter region of the Ras-like and estrogen-regulated growth inhibitor (RERG) gene. PGE2, via protein kinase A, phosphorylates RERG and enhances its nuclear translocation, inducing the proliferation of primary endometriotic cells (Monsivais et al., 2014). Another estradiol-regulated ESR2 target gene, serum and glucocorticoid-regulated kinase (SGK1), is also induced by PGE2 and contributes to survival of endometriotic cells via inhibition of apoptosis (Monsivais et al., 2016). The key role of ESR2 in proliferation and survival of endometriotic cells is further supported by studies in a mouse model of endometriosis expressing high levels of ESR2 in endometriosis lesions. The inhibition of ESR2 activity by an ESR2-selective estrogen antagonist suppresses lesion growth in these animals, whereas gain of ESR2 function stimulates the progression of endometriosis (Han et al., 2015). Mechanistically, this work also reveals that ESR2 interacts with components of the cytoplasmic inflammasome to increase IL-1β, thus contributing to cellular adhesion and proliferation (Han et al., 2015).

Endothelial cells in the human endometrium express ESR2 but not ESR1 (Crichtley et al., 2001; Tamura et al., 2004). In addition, estradiol
via ESR2 rapidly induces COX-2 expression in uterine endothelial cells (Critchley et al., 2001; Tamura et al., 2004). Thus, it is tempting to speculate that ESR2 may have a major role in regulating COX-2 levels in endometriosis. ESR2 signaling integrates inflammatory and pain pathways; macrophage infiltration of endometriosis increases in response to estradiol (Greaves et al., 2015). ESR2-immunoreactive macrophages contribute to inflammation by mediating nerve-growth in a mouse model (Greaves et al., 2015). Treatment of sensory neurons with diarylpropionitrile (DPN), an ESR2-selective estrogen agonist, results in upregulation of the capsaicin receptor, TRPV1, which may contribute to inflammation-associated pain perception in endometriosis (Caterina et al., 2000; Greaves et al., 2014).

A recently developed ESR2 ligand with anti-inflammatory properties, chloroindazole, prevents lesion establishment via suppression of inflammation, and inhibits angiogenesis and neurogenesis in a mouse model (Mallia et al., 2000). Moreover, the SRC-1 inhibitor bufalin, which leads to ESR2 protein degradation, induces apoptosis in endometriotic epithelial cells and inhibits proliferation of stromal cells (Cho et al., 2018). Agents that inhibit ESR2-mediated inflammation may be promising novel treatment options for endometriosis without interfering with fertility. Future preclinical studies are required to determine the efficacy and safety of these agents.

**Progesterone receptor**

In the endometrium, progesterone exerts its effects through intracellular PGRs PGR-A and PGR-B, both of which are encoded by a single gene (Kastner et al., 1990). PGR-A is a 94 kDa protein, whereas PGR-B is 114 kDa protein with 164 additional amino acids at its N-terminal (Lessey et al., 1983; Alexander et al., 1989). In mice, selective ablation of PGR-A results in uterine and ovarian abnormalities and female infertility, while ablation of PGR-B halts breast development only (Mulac-Jericevic et al., 2000, 2003). While PGR-A is sufficient for endometrial and ovarian function in mice, in humans, the roles of PGR-A and PGR-B seem to be more complex (Mote et al., 1999). Transcriptional activities of different PGR isoforms depend on the cell type as well as the promoter; e.g. in the endometrium, only PGR-A exerts repressor actions on ESR1 (Vegeto et al., 1993). Through PGR-A, progesterone acts like a brake on estradiol action in endometrial epithelium. It inhibits estrogen-induced growth and proliferation and can even reverse hyperplasia (Yang et al., 2011; Kim et al., 2013). This inhibitory action is mediated through stromal cells in a paracrine fashion, as PGR knockout endometrial tissue recompensants show that stromal PGR is essential for progesterone to antagonise estrogen-induced epithelial proliferation (Kurita et al., 2000). In response to progesterone, endometrial stromal cells also take up retinol and produce retinoic acid, which induces the enzyme 17β-hydroxysteroid dehydrogenase type 2 (HSD17B2) in endometrial epithelial cells, possibly in a paracrine fashion (Cheng et al., 2008). Thus, in endometrial epithelial cells, progesterone not only antagonises the action of estrogen but also induces HSD17B2, the enzyme that converts estradiol to the less potent estrone (Yang et al., 2001).

**Progesterone resistance in endometriosis**

The gene expression profile of endometriotic cells during the window of implantation reveals that progesterone-responsive genes such as that for glycodulin are significantly downregulated in women with endometriosis compared to endometrial cells from disease-free women (Kao et al., 2003). Further comparisons at various points during the menstrual cycle show dysregulated gene expression in the early secretory phase, with increased survival and mitotic activity (Burney et al., 2007). This attenuation of progesterone responsiveness can be explained by decreased PGR levels in endometriotic cells. Despite the ability of these cells to produce large amounts of progesterone through locally expressed steroidogenic enzymes, very low levels of PGR-A are present, while PGR-B is undetectable in endometriotic tissues obtained from peritoneal lesions (Attia et al., 2000). In eutopic endometrial tissue, both PGR isoforms increase throughout the proliferative phase and peak before ovulation under the influence of increasing estrogen levels; in endometriosis, PGR levels remain very low to undetectable, possibly due to an abnormally low ESR1: ESR2 ratio in endometrial stromal cells (Bulun et al., 2012).

Defective epigenetic programming may also contribute to progesterone resistance (Xue et al., 2007b). Endometrial mesenchymal stem cells (eMSC) isolated from the eutopic endometrium of women with endometriosis do not properly decidualise in vitro in response to hormone treatment; this suggests that progesterone resistance in endometriotic tissue may be inherited from defectively programmed stem cells (Barragan et al., 2016). A decreased progesterone response in mature stromal cells impedes HSD17B2 induction in epithelial cells, thus contributing to high levels of local estradiol by hindering its conversion into the biologically less active steroid estrone (Bulun et al., 2006).

**Mechanisms affecting PGR expression in endometriotic tissue**

Associations of PGR gene polymorphisms with endometriosis have been reported but results are inconsistent (Wieser et al., 2002; Lattuada et al., 2004; De Carvalho et al., 2007; van Kaam et al., 2007). A meta-analysis comparing 12 studies concluded that the link between a PGR variant and endometriosis risk was only observed in European subjects (Pabalant et al., 2014). Therefore, polymorphisms directly affecting PGR function do not likely explain the development of progesterone resistance in endometriotic tissue. The PGR-B promoter is hypermethylated in ectopic endometrial epithelium, which may suppress its expression in endometriosis (Wu et al., 2006). In fact, treatment of an immortalized endometriotic epithelial cell line with the pro-inflammatory cytokine TNF induces hypermethylation of the PGR-B promoter (Wu et al., 2008). Gene expression profiling in a baboon endometriosis model shows that as the disease progresses, a progesterone-resistant phenotype appears not only in the ectopic lesions but also in the eutopic endometrium (Afshar et al., 2013). These observations collectively suggest that inflammation may regulate PGR expression via epigenetic reprogramming.

Additionally, miR-196a, miR-29c and miR-194-3p have been shown to be associated with progesterone resistance. miR-194-3p hinders decidualization in endometrial stromal cells via direct regulation of PGR expression (Pei et al., 2018). miR196a downregulates PGR expression via the ERK/MEK pathway and inhibits decidualisation (Zhou et al., 2015). miR-29c, which is also overexpressed in eutopic endometrium of women with endometriosis and in baboon models, downregulates PGR by decreasing FK506-binding protein 4 (FKBP4).
levels (Yang et al., 2012; Joshi et al., 2017). Surgical excision of endometriosis lesions may help overcome the development of progesterone resistance by alleviating the inflammatory milieu (Joshi et al., 2017).

ESR2 binds to alternatively used promoters in the ESR1 gene and suppresses its expression in endometrial stromal cells (Trukhacheva et al., 2009). ESR1 suppression by high levels of ESR2 in endometriosis may thus hinder E2/ESR1-mediated induction of PGR in this tissue (Trukhacheva et al., 2009). It is possible that ESR2 may also directly bind to PGR promoters to suppress its transcription.

Retinoic acid signaling and function in the endometrium

Retinoic acid is traditionally known to have anti-inflammatory effects (Schug et al., 2007). Transcriptional activation through RARs may trigger differentiation, cell-cycle arrest and apoptosis (Altucci et al., 2001; Kitareewan et al., 2002; Donato and Noy, 2005). Alternatively, retinoic acid can activate transcription to promote cell survival through an orphan NR, peroxisome proliferator-activated receptor beta/delta (PPAR-β/δ) (Shaw et al., 2003). Shuttling of retinoic acid to the NRs is regulated by the intracellular lipid binding proteins, cellular retinoic acid-binding protein 2 (CRABP2) and fatty acid-binding protein 5 (FABP5), which transfer retinoic acid to RAR or PPAR-β/δ, respectively (Tan et al., 2002; Schug et al., 2007). Thus, the ratio of CRABP2 and FABP5 in a particular cell regulates retinoic acid signaling to exert either pro-apoptotic or pro-survival effects (Schug et al., 2007; Çakıroğlu et al., 2017).

Retinoic acid production and metabolism are crucial for endometrial decidualisation and the crosstalk between stroma and the glandular epithelium. Progesterone via PGR induces retinoic acid production in endometrial stromal cells, which then acts on epithelial cells to induce HSD17B2 expression (Casey et al., 1994; Cheng et al., 2008). In endometriotic tissue, defective retinol uptake and retinoic acid metabolism resulting from progesterone resistance compromises the crosstalk between the stroma and epithelium (Çakıroğlu et al., 2017).

Retinoic acid deficiency

Retinol uptake and retinoic acid action are impaired in endometriotic stromal cells collected from ovarian endometriomas (Pavone et al., 2010). The cell surface receptor stimulated by retinoid acid 6 (STRA6), which is the main receptor for retinol uptake by endometrial stromal cells, is downregulated in endometriotic stromal cells compared to normal endometrial stromal cells (Pavone et al., 2011). Impaired intracellular conversion of retinol to its active form, all-trans retinoic acid, and deficiency of retinol binding protein 1 (RBPl) also contribute to decreased availability of retinoic acid (Pavone et al., 2011; Pierzchalski et al., 2014). Moreover, endometriotic stromal cells express the oxidizing enzyme CYP26B1 at higher levels, which causes a more rapid clearance of retinoids (Pavone et al., 2017). Decreased availability of retinoic acid gives rise to deficiency of HSD17B2 in the epithelial compartment. HSD17B2 deficiency impairs the conversion of potent estradiol to its less active form estrone, further contributing to the hyperestrogenic environment in endometriosis (Pavone et al., 2011).

Anti-apoptotic effects of altered retinoid metabolism

Retinoic acid deficiency can enhance cell survival pathways in endometriosis via at least two different ways. First, decreased retinoic acid levels lead to increases in inflammatory cytokines such as IL-6 (Wang et al., 2007). In an immunocompetent mouse model, retinoic acid was shown to inhibit the development of peritoneal endometriotic implants while reducing IL-6 and monocyte chemoattractant protein 1 levels and increasing macrophage differentiation (Wieser et al., 2012). Second, retinoic acid deficiency can lead to cell growth and protect against apoptosis due to a switch in cell signaling pathways from CRABP2/RAR to FABP5/PPAR-β/δ (Schug et al., 2007). CRABP2 expression is induced by progesterone, and progesterone resistance in endometriosis severely decreases the CRABP2:FABP5 ratio (Pavone et al., 2010). FABP5 dominancy promotes the shuttling of retinoic acid to PPAR-β/δ, which promotes cell survival and impairs apoptosis (Pavone et al., 2010). Recently, it was shown that expression of the retinoid acid-regulated autophagy marker Beclin1 is reduced in endometriotic tissue compared to endometrium, and attenuation in autophagy may contribute to enhanced endometriotic stromal cell proliferation (Lu et al., 2018). Collectively, decreased availability of retinoic acid and a switch in its intracellular signaling may favor anti-apoptotic pathways and cell survival in endometriosis.

Endometrial stem cells

The endometrium undergoes cyclical regeneration in response to the steroid hormones estradiol and progesterone (Jabbour et al., 2006). The extraordinarily high regenerative capacity of this dynamic tissue led to the search for stem cell-like endometrial cell populations, characterized by the capacity for colony formation, self-renewal and differentiation. Rare stromal and epithelial cells (1.25 and 0.22%, respectively) with high proliferative capacity were identified, providing the first evidence of endometrial stem cells (Chan et al., 2004; Schwab et al., 2005). Following these reports, several putative endometrial stem cell populations were identified including side population cells, eMSCs and bone marrow-derived mesenchymal stem cells (BMDSCs) (Gargett et al., 2016).

The side population phenotype is a universal marker for adult stem cell activity in various tissues (Challen and Little, 2006). Side population cells express ATP-binding cassette transporter (ABCG2/Bcrp1) on the cell surface and have the ability to efflux the DNA binding dye Hoechst 33342 (Zhou et al., 2001). These perivascular cells comprise ~2% of the human endometrium distributed in the functionalis and the basalis layers and display an endothelial-cell phenotype with migration and angiogenesis abilities (Masuda et al., 2010). When injected into subcutaneous tissue or the kidney capsule of NOD-SCID mouse, side population cells regenerate human endometrial stroma, glandular epithelium and endothelium (Cervello et al., 2010, 2011; Miyazaki et al., 2012). While the side population may harbor endometrial stem cells, this population is heterogeneous and isolation techniques are impractical for further applications due to isolation-associated toxicities (Cousins et al., 2018).
eMSCs are self-renewing, multipotent, clonogenic stem cells that can give rise to mesodermal lineages in vitro. eMSCs were first isolated from the endometrium as CD146(+)PDGFRB(+) dual positive cells and later by a novel single cell surface marker, sushi domain containing-2 (SUSD2) (Gargett and Masuda, 2010). Similar to the side population, eMSCs are located in the perivascular region and they are present in both functionalis and basalis layers (Schwab and Gargett, 2007; Masuda et al., 2012). Comparison of post-menopausal and premenopausal endometrial tissues revealed eMSCs with similar self-renewal capacity and expression of SUSD2 (Ulrich et al., 2014).

In addition to endogenous stem cells of the endometrium, BMDSCs may also contribute to endometrial regeneration. Endometrial biopsy of HLA-mismatched bone marrow donor recipients demonstrated donor-derived cells in the stroma and the epithelium (Taylor, 2004; Ikoma et al., 2009). BMDSCs were also shown to serve as progenitor cells for endometriotic lesions (Du and Taylor, 2007). Later studies suggested that BMDSCs may differentiate into stroma or epithelium but do not contribute to the endometrial progenitor population (Cervello et al., 2012). Recently, a study of a chimeric irradiated mouse model transplanted with immunofluorescent labeled marrow showed that bone marrow-derived cells that infiltrate into the epithelium and vascular compartments may be F4/80(+) macrophages (Ong et al., 2018). As macrophages secrete vimentin and are weakly positive for leukocyte marker CD45, they could easily be mistaken for endometrial cells (Mor-Vaknin et al., 2003; Ong et al., 2018). Thus, recent evidence suggests that BMDSCs may not contribute to endometrial regeneration via trans-differentiation. Regardless, BMDSCs seem to enhance endometrial regeneration. Endometrial injury increases the engraftment of these cells to the tissue independent of sex steroid hormones (Du et al., 2012). Cell therapy with autologous peripheral blood CD133+ BMDSCs significantly improves endometrial thickness and the duration and intensity of menstruation and decreases adhesion scores in patients with refractory Asherman syndrome (Santamaria et al., 2016). Thus, it is conceivable that BMDSCs may improve endometrial regeneration via secreting paracrine factors to stimulate endogenous endometrial stem cells.

**Stem cells in endometriosis**

Based on clinical and molecular evidence, retrograde menstruation is the most plausible theory explaining the development of endometriosis, with some of the endometrial tissue effluxed to the peritoneum during menstruation (Sampson, 1927; Bulun, 2009). Yet, it is not known why endometriosis occurs only in 10% of women while retrograde menstruation through the fallopian tubes occurs in ~90% of women (Halme et al., 1984). Therefore, predisposing factors are likely involved, either in the shed endometrial cells that enable survival in the peritoneal cavity or in the immune response that leads to impaired clearance of the migrated cells.

Similar to endometrium, endometriotic lesions also harbor multipotent, colony-forming MSCs (Chan et al., 2011). Remarkably, MSCs and epithelial progenitor cells isolated from ovarian endometriomas do not show increased clonogenicity or self-renewal compared to endometrial stem cells, which might have explained the increased survival capacity in endometriosis (Chan et al., 2011; Li et al., 2014). However, because in-vitro culture lacks the paracrine factors unique to the stem cell niche, it is unable to recapitulate normal physiology. It was shown that cytokines such as colony-stimulating factor-1 secreted from macrophages increase the proliferation and invasion activity of endometriotic stem cells (Chan et al., 2017). Other molecular qualities of endometriotic stem cells, such as aberrant NR expression, could also give rise to a resilient phenotype.

**NRs in endometrial stem cells**

Endometrial side population cells are ESR2-positive, but they do not express ESR1 or PGR (Cervello et al., 2011). Similarly, SUSD2(+) eMSCs are also ESR1-negative (Ulrich et al., 2014). Based on their perivascular location, it is tempting to speculate that these MSCs represent the first cell type that comes into contact with steroids and growth factors transported by blood. These stem cells may respond to circulating estradiol primarily via ESR2, the predominant estrogen receptor type. In post-menopausal women, estradiol treatment increases the total number of SUSD2(+) eMSCs and the endometrial thickness proportionally (Ulrich et al., 2014). This proliferation in response to estradiol could be due to ESR2-mediated growth or paracrine signaling between ESR1/PGR-positive mature stromal cells and neighboring stem cells.

The proposed stem cell signaling theory for leiomyomas can be taken as a model for the endometrium. For the stimulation of clonal expansion and growth of leiomyoma, sex steroids and steroid hormone receptors are necessary; yet, leiomyoma stem cells express barely detectable levels of ESR1 or PGR (Mas et al., 2012; Ono et al., 2012). The suggested mechanism for hormone action on leiomyoma stem cells proposes that differentiated stromal cells respond to circulating steroid hormones by secreting paracrine factors, which in turn stimulate stem cell proliferation and tumor expansion (Bulun, 2013). Supporting this model, it was recently shown that a PGR-positive group of cells in uterine leiomyomas secrete receptor activator of nuclear factor kappa-B ligand (RANKL) following progestin treatment, which induces proliferation of leiomyoma stem cells through its receptor RANK (Ikhena et al., 2018). Endometrial or endometriosis stem cells, which are also deficient in steroid receptors, may also be stimulated via unique paracrine signaling originating from the surrounding steroid receptor-positive cells.

Epigenetically programmed populations with stem cell characteristics may be present in eutopic endometrial tissue of women predisposed to develop endometriosis. These cells may inappropriately express high levels of pro-inflammatory (e.g. ESR2) or steroidogenic (e.g. NR5A1) factors that increase their capacity to survive outside of the uterine cavity. Upon arrival in the peritoneal cavity via retrograde menstruation, these cells would be more likely to form endometriotic lesions (Fig. 2). Thus, a combination of defective epigenetic programming combined with larger numbers of cells with stem cell characteristics could constitute a significant risk factor for developing the symptoms of pelvic endometriosis.

**Novel therapeutic approaches targeting steroid receptors**

Current available medical treatment options for endometriosis include combined oral contraceptives, gonadotropin-releasing hormone agonists...
Molecular characteristics of steroid hormone receptors in endometriotic tissue. In endometriotic tissue, high levels of local estradiol are maintained by the upregulation of various nuclear receptors such as NRS1A and ESR2, which are connected via feedback loops involving inflammatory factors. The abnormally low ESR1:ESR2 ratio in endometriotic stromal cells is associated with low levels of PGR, which leads to progesterone resistance and further contributes to a hyperestrogenic environment via an abnormal retinoid acid signaling pathway. COX-2, cyclooxygenase-2; ESR1, estrogen receptor alpha; ESR2, estrogen receptor beta; HSD17B2, 17β-hydroxysteroid dehydrogenase type 2; PGE2, prostaglandin E2; PPARβ/δ, peroxisome proliferator-activated receptors beta/gamma, PGR, progesterone receptor; RAR, retinoic acid receptor; RERG, RAS-like estrogen-regulated growth inhibitor; NRS1A, steroidogenic factor 1; StAR, steroidogenic acute regulatory protein; TNF, tumor necrosis factor.

Figure 3

Figure 2 Role of endometrial stem cells in endometriosis pathogenesis. Normal mature endometrial cells that are shed in the peritoneal cavity through retrograde menstruation fail to attach and are absorbed. Epigenetically defective endometrial cells with stem cell characteristics display enhanced cell survival and lead to lesion formation. These endometriotic cells have hypomethylated CpG islands at promoters that regulate genes encoding critical nuclear receptors such as ESR2 and NRS1A. These nuclear receptors alter estrogen production and action and also the response to progesterone.

and antagonists, oral or injectable progestins and aromatase inhibitors (Dunselman et al., 2014). Almost all available agents result in only temporary symptom relief (Bedawy et al., 2017). Since the basis of therapy is ovulation interruption and estrogen suppression, non-steroidal anti-inflammatory drugs are the only treatment option for women who wish to maintain fertility. In addition, there is no evidence to show that pregnancy outcomes improve following ovulation suppression therapy for endometriosis (Hughes et al., 2007). Thus, novel therapeutic approaches with better sustainable outcomes and higher tolerability are needed.

Since estradiol is known to promote proliferation of the endometrium, several studies have tested selective estrogen receptor modulators (SERMs) to drive endometriotic lesion regression. Raloxifene and bazedoxifene both result in lesion regression and inhibition of proliferation in murine models (Yao et al., 2005; Kulak et al., 2011; Naqvi et al., 2014). Nonetheless, a prospective randomized clinical trial of post-excision raloxifene for biopsy-proven endometriosis showed that those who received raloxifene following surgical lesion excision experienced pain sooner than the placebo arm and raloxifene did not inhibit lesion growth (Stratton et al., 2008). This discrepancy may have several possible explanations. First, while ESR2 mediates estradiol-dependent anti-apoptosis and proliferation in human lesions, its expression is variable in murine models of endometriosis with ESR1 dominance in most models (Kulak et al., 2011; Naqvi et al., 2014). Second, raloxifene decreases proliferative markers in the epithelium of lesions in rodent models but not in the stroma, yet the stromal component is the major contributor to endometriotic lesions (Altintas et al., 2010). Finally, estradiol agonism or antagonism may have direct effects on nociceptors or pain perception. ESR2 ligands acting as estrogen antagonists in endometriotic tissue show promising results in murine models and should also be considered as potential therapeutics (Zhao et al., 2015).

Selective PGR modulators (SPRMs) are synthetic steroids that exert agonistic and antagonistic properties upon binding to PGR in a tissue-specific manner (Madauss et al., 2007). SPRMs induce amenorrhea without causing a hypoestrogenic state or associated bone loss (Chabbert-Buffet et al., 2005). These agents also reduce abnormal uterine bleeding that is observed with progestin treatment (Donnez et al., 2015). SPRMs that primarily act as progesterone antagonists are ulipristal acetate (UPA), asoprisnil and mifepristone. UPA decreases lesion size, attenuates cell proliferation, and induces apoptosis in murine endometriosis models (Huniadi et al., 2013; Liang et al., 2018). Mifepristone also reduces lesion size in animal models and patients (Kettel et al., 1996; Mei et al., 2010). In parallel with these findings, a systematic review of 10 randomized controlled trials assessing the efficacy of SRPMs for relief of endometriosis-associated pain concluded that mifepristone relieves dysmenorrhea and dyspareunia, with insufficient evidence of efficacy and safety of other SPRMs (Fu et al., 2017). SPRMs may be a promising new type of medication for clinical use, but their long-term efficacy remains to be proven (Whitaker et al., 2014).

Discussion

A woman with first degree relatives with endometriosis is seven times more likely to develop endometriosis compared to those who have unaffected relatives (Simpson et al., 1980). Despite some evidence that endometriosis may be an inherited disease, no germ-line or causative somatic mutations have been reported to date (Montgomery et al., 2008; Rahmioglu et al., 2012). While nucleotide variants or mutations were noted sporadically in the epithelial component of ovarian endometriomas or deep-infiltrating endometriosis, stromal cells consistently lack any somatic mutations (Anglesio et al., 2017). Epigenetic aberrations in endometriotic stromal cells seem to be the main contributors to the pathogenesis and progression of
endometriosis (Dyson et al., 2014; Kokcu, 2016; Koukoura et al., 2016). Among these epigenetic abnormalities, differential methylation of the ESR2 promoter seems to have a crucial impact. The shift from ESR1 to ESR2 dominance changes the estradiol responses and blunts estradiol induction of PGR in stromal cells, leading to reduced expression of PGR isoforms. The decreased ESR1:ESR2 ratio likely reshapes eMSC chromatin organization, giving rise to an acquired heritable progesterone resistance. Repeated exposure of the peritoneum to epigenetically defective eMSCs via menstruation precipitates formation of endometriotic lesions. Following lesion formation, estrogen via ESR2 and pro-inflammatory factors increase inflammation and survival of endometriotic cells within the lesion.

The ability of endometriotic stromal cells to express the full set of steroidogenic enzymes as well as sulfatase pathway enzymes results in remarkable amounts of local estradiol production (Rizner, 2016). Inflammation and increased PGE2 induce steroidogenic enzymes, including aromatase and StAR, via translocation of NR5A1 to their promoter regions, which competes with NR2F2. Activation of this cascade back with ESR2 through increased local estradiol, further reinforcing inflammation by increasing COX-2 levels, and creating a vicious cycle (Fig. 3). It is important to note that not only the interaction between hormone receptors and sex steroids, but also the change in hormone levels during the menstrual cycle, are the key factors of this phenotype. SPRMs seem to be effective in alleviating endometriosis-associated pain without creating a hypoestrogenic state, suggesting that repeated menstruation and ovulation may be more critical to reinforce this inflammatory environment than continuous estrogen exposure. It is possible that de-escalating the inflammatory environment will lead to epigenetic reprogramming of stem cells and help overcome progesterone resistance in the long term. New synthetic ligands targeting ESR2, PGR and RAR are promising future treatments for endometriosis. Expanding our understanding of the NR signaling mechanisms in relation to hormonal fluctuations could guide development of other novel non-hormonal therapeutic agents without disturbing fertility.

**Authors’ roles**

B.D.Y. performed the literature search and wrote the initial draft of the article. S.E.B. revised the article. Both authors reviewed and approved the submission of the article.

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**Conflict of interest**

The authors declare they have no conflicts of interest.

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