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Progesterone resistance in endometriosis: origins, consequences and interventions

Running headline: **Progesterone resistance in endometriosis**

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Abstract
Endometriosis is a common cause of pelvic pain and affects up to 10% of women of reproductive age. Aberrant progesterone signaling in the endometrium plays a significant role in impaired decidualization and establishment of ectopic endometrial implants. Eutopic endometrial cells from women with endometriosis fail to downregulate genes needed for

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decidualization, such as those involved in cell cycle regulation, leading to unbridled proliferation. Several causes of progesterone resistance in the endometrium have been postulated, including congenital "preconditioning", whereby the in utero environment renders infants susceptible to neonatal uterine bleeding and endometriosis. Progesterone action is crucial to decreasing inflammation in the endometrium, and deviant progesterone signaling results in a proinflammatory phenotype. Conversely, chronic inflammation can induce a progesterone resistant state. Repetitive retrograde endometrial shedding begets chronic peritoneal inflammation, which further exacerbates progesterone resistance. Genetic causes of progesterone resistance include progesterone receptor gene polymorphisms, altered microRNA expression, and epigenetic modifications to progesterone receptors and their targets. Environmental toxins, such as dioxin, play a possible role in the genesis of endometriosis by permitting an inflammatory milieu. A consequence of impaired progesterone action is that hormonal therapy is rendered ineffective for a subset of women with endometriosis. Synthetic progestins, such as dienogest, may overcome this phenomenon by increasing progesterone receptor expression and decreasing pro-inflammatory cytokines. Other modalities include high dose depot formulations of progestins, medicated intrauterine devices and the likely advent of oral GnRH antagonists. Unearthing root causes of progesterone inaction in endometriosis will aid in development of novel therapeutics geared toward prevention and treatment.

Keywords

endometriosis, progesterone, endometrium, progesterone receptor, infertility, pelvic pain,

Abbreviations

17βHSD2, 17β hydroxysteroid dehydrogenase 2 gene
AHR, arylhydrocarbon receptor
BCL2, B-cell lymphoma 2 protein gene
CB1-R, cannabinoid receptor type 1
CRABP2, cellular retinoic acid binding protein 2
Cx43, connexin 43
DNG, Dienogest: 17α-cyanomethyl-17β-hydroxyestra-4,9-dien-3-one
eMSCendo endometrial mesenchymal stem cells

ER, estrogen receptor

eSFendo, endometriosis stromal fibroblasts

FABP5 fatty acid-binding protein, epidermal

FOXO1, forkhead box protein O1

GnRH, gonadotropin releasing hormone

HOXA10, homeobox A10

IL, interleukin

LNG-IUS, levonorgestrel intrauterine system

miR, microRNA

MMP, matrix metalloproteinase

NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells

PPARβ/δ, peroxisome proliferator activated receptors β and δ

PR, progesterone receptor

PR-A progesterone receptor A isoform

PR-B progesterone receptor B isoform

RA retinoic acid

RANTES, regulated upon activation, normal T cell expressed and secreted or CCL5

STRA6 vitamin A receptor

TGF-β2, transforming growth factor-beta 2

TNF-α, tumor necrosis factor-α

TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin

Key message

Endometriosis is caused, in part, by loss of progesterone signaling in eutopic and ectopic endometrial tissues. Elucidating the etiology of progesterone inaction will enable formulation of effective therapies directed towards symptom management and prevention.

Introduction

Progesterone (preg-4-ene-3,20-dione) is a natural cyclopentanoperhydrophenanthrene cholesterol catabolite produced abundantly in the corpus luteum. This ovarian structure was recognized since the early 1900s as the glandular source of "internal secretions" necessary for establishment and maintenance of pregnancy. In 1929, George Corner and Willard Allen successfully isolated progesterone from corpora lutea at the University of Rochester (1) and the structure of purified progesterone was subsequently deciphered in 1934. An equally

fascinating saga of synthetic progestin production, revolutionized by Russell Marker's contemporaneous discovery that diosgenin, a sterol derived from Mexican yams, could be chemically transformed into progesterone, led to the development of synthetic pharmaceutical progestins. Progesterone is the quintessential "pro-gestational" hormone, maintaining pregnancy by promoting decidual vascularization and myometrial quiescence. Arpad Csapo, a protégé of Corner, was one of the early proponents of the progesterone "block" theory, whereby progesterone can reversibly inhibit uterine muscle contractility. In effect, labor can be considered a "progesterone (P4) resistant state" and recent findings support the idea that functional progesterone withdrawal can be achieved by alterations in the relative expression of the truncated (by 164 N-terminal amino acids) progesterone receptor (PR) A isoform (PR-A) to the longer B isoform (PR-B), the former serving as a trans-repressor of the latter (2). Progesterone also plays critical roles in endometrial differentiation, uterine fibroid growth and breast carcinogenesis. In this review, we will summarize evidence in support of the hypothesis that endometriosis tissues are progesterone-resistant (3) and how this molecular defect might be restored to alleviate the primary symptoms, pelvic pain and infertility, associated with this common disorder.

Evidence of progesterone resistance in endometriosis

The first published evidence that supported the concept of progesterone resistance was the finding by Attia et al. that the receptor PR-B mRNA and protein levels were significantly reduced in endometriosis lesions, whereas PR-A isoforms were generally spared (4). Subsequently, a series of endometrial gene expression microarray studies indicated that progesterone-regulated genes (eg, glycodelin, N-acetylglucosamine-6-O-sulfotransferase, 17β hydroxysteroid dehydrogenase 2 [*17βHSD2*]) were downregulated in tissues derived from endometriosis subjects compared to women without laparoscopic evidence of endometriosis (5).

As indicated above, a decrease in expression of *17βHSD2* has been noted in the eutopic endometrium in numerous studies of endometriosis (5). This enzyme plays a key role in the conversion of biologically active estradiol to the less potent estrone, and a consequence of its decreased expression is enhanced estradiol activity within endometriotic lesions, even in the presence of progestin therapy. The inability of endometriotic lesions to upregulate *17βHSD2* in response to progesterone may be due to decreased expression of PR-B in stromal cells of this tissue, thereby rendering estradiol activity unchecked in these lesions.

That eutopic endometrium in women with endometriosis exhibits aberrant gene expression in response to the sex steroids has been well-established. The enhanced secretion of progesterone after ovulation is required for the downregulation of genes associated with DNA replication – a mechanism by which endometrial proliferation is rapidly halted in the early secretory phase. Genes involved in cell cycle regulation, such as proliferating cell nuclear antigen (*PCNA)*, cellular marker of proliferation (*Ki67*), thymidine kinase 1, cyclin E1 protein (*CCNE1*), forkhead box protein O1 (*FOXO1*) and mitotic arrest deficient-like 1 protein (*MAD2L1*), are downregulated in the early secretory phase in women without disease in response to progesterone and upregulated in patients with moderate-severe disease (5). Similarly, expression of mitogen-induced gene 6 (*MIG6*), a negative regulator of epidermal growth factor (EGF) signaling, is significantly decreased in the endometrium of patients with endometriosis. Conversely, expression of anti-apoptotic genes, such as B-cell lymphoma 2 protein *(BCL2)*, are aberrantly increased in the early secretory endometrium of patients with endometriosis, signifying a failure of progesterone to rapidly halt endometrial proliferation and induce a differentiated state (5). The roles of micro- (miR) and non-coding RNAs are discussed below.

Incomplete endometrial decidualization and failure to suppress cell cycle genes are likely key factors in impaired implantation, a factor increasingly recognized in endometriosis-associated infertility (3,5). Putative characteristics of the window of implantation include upregulation of progesterone target genes that facilitate embryo attachment and regulate the local immune response. Genes such as *MUC-1* and osteopontin are important for embryo attachment and are upregulated in normal women but downregulated in patients with endometriosis (5). Similar findings have been noted for glycodelin, an immunomodulator regulated by progesterone, which is downregulated during the window of implantation in decidua of patients with endometriosis (3,5). Impaired decidualization in cells from patients with endometriosis is further evidenced by a 2-fold decrease in expression of insulin-like growth factor binding protein-1 (IGFBP1) and prolactin, which are usually expressed robustly in secretory endometrium of patients without endometriosis (6). In vitro studies have also exhibited a diminished capacity of progesterone to suppress expression of matrix metalloproteinases (MMP), specifically MMP-3 and MMP-7, in stromal cells from patients with endometriosis (7). We reported that production of the gap junction protein connexin 43 (Cx43), typically regulated in endometrial stromal cells by luteal hormones, is reduced ~50% in endometria from women with endometriosis. A consequence of low Cx43 expression is

that stromal cell-to-cell communication is disrupted and the decidua fails to adopt a phenotype ideal for embryonic receptivity (8).

The exact mechanism(s) by which progesterone response is attenuated in patients with endometriosis remain(s) unclear. Some studies show an inadequate rise in systemic progesterone levels (9), while others show no differences (10). This resistance also may be evidenced by altered expression of the nuclear progesterone receptor isoforms PR-A and PR-B, steroid receptor coactivators, and numerous downstream effectors (2,5). Furthermore, when gonadotropin releasing hormone (GnRH) agonist-suppressed healthy women were supplemented with exogenous progesterone, no significant differences in endometrial histology were observed, even at low serum progesterone levels. Hence, impaired progesterone action in the endometrium of patients with endometriosis is likely attributable to inherent progesterone resistance of the endometrium itself and not to impaired progesterone secretion (11).

Causes of progesterone resistance in endometriosis

Congenital

A recent hypothesis suggests that endometriosis and progesterone resistance in adult women may be a consequence of maternal and neonatal "preconditioning," wherein newborn progesterone resistance persists through early adolescence (12). Histochemical analyses of neonatal endometria reveal a spectrum of progesterone responses, ranging from partial or complete resistance in the majority (67%) to full responses in a minority (33%) (13). Newborn menstruation is a well-known phenomenon, with overt cases ranging from 3-6% and occult biochemical evidence ranging from 25-61% (14), occurring as a result of withdrawal of placental steroid hormones postpartum. Due to its relatively long canal length and inspissation of cervical secretions, the neonatal cervix is effectively occluded from the 26th week of gestation. In line with Sampson's theory, retrograde efflux of endometrial cells after delivery may serve as a nidus for establishment of endometriosis lesions in the neonate. Indeed, the presence of endometriosis has been documented in necropsy studies of human female neonates (15). Intrauterine growth restriction is recognized as a risk factor for both neonatal uterine bleeding and endometriosis. A case-control study of 743 women who underwent laparoscopy found that those with birth weights <2500gms had a significantly higher prevalence of endometriosis than those with birth weights >2500gms (16). Other epidemiological studies have concluded that low birth weight increases the likelihood of

future endometriosis, with a relative risk = 1.2 (95% CI 1.0–1.8, $p<0.01$) (17). Post-maturity has also been described as a significant risk factor for the development of endometriosis as the incidence of newborn uterine bleeding appears to be higher in these neonates.

Inflammation

Considerable evidence shows a link between progesterone resistance and chronic inflammatory states among patients with endometriosis. As indicated above, progesterone action in the secretory endometrium primarily serves to halt estrogen-driven endometrial proliferation, elicits differentiation of the endometrium, and recruits specialized immune cells to support embryonic implantation (18). Declining progesterone levels in the absence of pregnancy lead to an increase in local pro-inflammatory cytokines, chemokines, and MMPs, which then activate tissue break-down and menstruation. The anti-inflammatory properties of progesterone in uterine cells have been well-enumerated (2). Progesterone suppresses signaling by members of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) family of proteins in endometrial cells. This signaling network has been implicated in endometriosis as a factor leading to the establishment and maintenance of endometriosis implants (19). NF-kB proteins are transcription factors with the ability to control genes related to cell proliferation, adhesion, apoptosis, and inflammation. Hence, progesterone resistance leads to increased NF-kB activity, and a proinflammatory state. Ectopic implants express increased levels of proinflammatory cytokines, which increase NF-kB expression and, in turn, decrease PR expression and progesterone action, thereby establishing a proliferative state with attenuated differentiation.

Other cytokines also play vital roles in progesterone resistance through alteration of PRs. Tumor necrosis factor (TNF)-α and interleukin (IL)-1β directly decrease levels of both PR isoforms possibly via epigenetic modifications. For example, TNF-α exposure leads to promoter hypermethylation of the PR-B isoform in endometriotic cells, causing a decrease in expression and increase in PR-A/PR-B ratio (20). Proinflammatory cytokines may also disrupt receptor function through alterations in steroid receptor chaperone proteins, such as the immunophilin FK506 binding protein 5 (FKBP5), and receptor coactivators such as hydrogen peroxide-inducible clone 5/androgen receptor coactivator 55 (HIC-5/ARA55) (21). Other mechanisms by which proinflammatory cytokines disrupt PR function are through direct competition for receptor coregulators or interference between the functional bridges connecting PRs and other transcription factors, such as FOXO1, essential for expression of

key PR target genes (18). PR function can be modulated in a cell- and context-specific manner by a host of posttranslational modifications, including serine phosphorylation, ubiquitination and sumoylation that, in turn, affect their stability, half-life, trafficking, transcriptional activity and target gene selectivity (2).

Retrograde menses

Retrograde menses per se may induce a state of progesterone resistance, in part via peritoneal inflammation. In a nonhuman primate model of surgically-induced endometriosis, wherein menstrual endometrium is operatively transplanted into the pelvic cavity of healthy baboons, the pattern of eutopic endometrial gene expression was altered (22). While some genes remained dysregulated over the 16-month course of these experiments, others appeared to autocorrect after acute, transient upregulation (e.g., c-fos, which has been postulated to inhibit PR-mediated gene expression). This finding suggests that repetitive, cyclical retrograde menses may be necessary to sustain a chronic inflammatory environment within the peritoneal cavity to effect long-standing progesterone resistance. Alternatively, women and nonhuman primates with spontaneous endometriosis may carry populations of endometrial cells that are intrinsically insensitive to progesterone, as some of the other hypotheses discussed above would indicate.

Dioxin

The role of environmental toxicants in the genesis of endometriosis is of significant interest. Polycholorophenyls, particularly dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]) are postulated to instigate progesterone resistance and endometriosis (23). Dioxin-like compounds are persistent organic pollutants that enter the environment as byproducts of agricultural pesticides and waste incineration. These compounds are resistant to degradation, and bioaccumulate, especially in food sources (24). Much of the evidence to date implicating these compounds in the development of endometriosis has been anecdotal and initially was based on the fact that Belgium was found to have both a very high incidence of endometriosis as well as high levels of TCDD contamination. Due to the propensity of this lipophilic compound to accumulate in fat-rich tissues in the human body, breast milk can contain high levels of TCDD (23). Nonhuman primate models corroborate correlations of endometriosis with TCDD exposure. One study found a significantly increased incidence of endometriosis in rhesus monkeys exposed to TCDD for four years (25).

Biologic effects of TCDD are mediated through binding to the arylhydrocarbon receptor (AHR), and this ligand-receptor complex then binds to specific dioxin response elements to alter transcriptional activity of specific genes (23). Some studies have unearthed that a possible mechanism by which TCDD exposure may lead to endometriosis, through induction of progesterone resistance in the endometrium. Dioxins have been implicated in pregnancy loss via disruption of ovarian steroidogenesis and interference with progesterone action in the endometrium (26). In human endometriosis cells, proinflammatory chemokines that are typically downregulated by progestins (e.g., regulated upon activation, normal T cell expressed and secreted (RANTES) or CCL5) can be directly activated by dioxin-AHR complexes (27). In mouse models, TCDD exposure at all stages of development leads to loss of PR expression in the endometrium (26). A key mediator of progesterone action in the endometrium is transforming growth factor-beta 2 (TGF-β2), and immunoblot and immunohistochemical analyses reveal lower levels of TGF-β2 in endometrial cells of mice exposed to TCDD. Furthermore, TCDD exposure prevents progesterone-mediated downregulation of MMP expression and blunts its anti-inflammatory responses, possibly due to a decreased cannabinoid receptor type 1 (CB1-R) mRNA expression (28). Endometrial CB1-R expression normally increases in the secretory phase in response to progesterone and modulates immune responses required for establishment of pregnancy. Epigenetic changes in the PR promoter have been attributed to TCDD exposure, with a murine study showing methylation increased by 40%-60% after TCDD (29).

Mesenchymal Stem Cells

Since endometrial mesenchymal stem cells serve as progenitors to endometrial stromal fibroblasts, progesterone resistance in endometriosis may be acquired from these cells (30). In vitro studies have shown that stromal fibroblasts from patients with endometriosis (eSFendo) differentiated from endometrial mesenchymal stem cells (eMSCendo) exhibit progesterone resistance and a failure of decidualization, compared to cells from normal controls (eSFcontrol). Interestingly, while exhibiting a classic phenotype of progesterone resistance, eSFendo cells derived from in vitro cultured eMSCendo did not exhibit a pro-inflammatory phenotype, unlike eSFendo cells cultured directly from tissue. By contrast, eSFendo cells cultured directly from tissue demonstrated upregulation of a myriad of pro-inflammatory genes, including CXC motif chemokine 12 (*CXCL2), IL-8* and *MM*Ps, unlike eSFendo cells derived in vitro from eMSCendo cells. Hence, while progesterone resistance in eSFendo appears to be an inherited trait from progenitor cells, the pro-inflammatory

phenotype appears to be acquired after differentiation. These findings underscore the importance of cellular context- in this case, the "endometrial niche"- in the transition of eSFendo cells to those with an inflammatory phenotype, exacerbating progesterone resistance in vivo (30).

Genetics

A genetic basis of endometriosis is evidenced by increased incidence in identical twins and first degree family members (31). Several interesting endometriosis-related genes have been discovered via linkage and genome wide association methods. Some candidate genes have yielded promising insights, including genes coding for the estrogen receptor (ER), inflammatory cytokines and adhesion molecules, although an unequivocal consensus genetic "fingerprint" has not been reached thus far (32,33). Relevant to the question of progesterone resistance is the association of a progesterone receptor gene polymorphism and susceptibility to endometriosis (34). While several polymorphisms have been described in the PR gene, the *PROGINS* polymorphism has the potential to affect ligand-binding and downstream signaling in the cellular context of endometriosis, and is implicated as a genetic cause of progesterone resistance (35). The *PROGINS* polymorphism consists of three parts: 1. a 306 bp Alu insertion in intron 7/G, 2. a silent point mutation in exon 5 (*H770H*), and 3. a single amino acid change in exon 4 (valine 660 to leucine) (*V660L*). Wieser and colleagues first reported an increased frequency of the 306bp Alu insertion *PROGINS* polymorphism in patients with endometriosis using PCR and gel electrophoresis, with a reported frequency of 0.17 in women with endometriosis compared to a frequency of 0.08 in controls (34). Homozygosity of this particular allele was 0.03 in women with endometriosis compared to 0.01 in controls. A recent metanalaysis pooling 12 studies and involving 1,323 cases and 1,998 controls found a trend between the presence of a variant allele and risk of endometriosis, with a conferred risk odds ratio = 1.41–1.43 (p=0.15–0.17) in homozygous and recessive models (35), but the association was only observed in European subjects.

Epigenomics and Epigenetics

Epigenetic modifications to the transcriptional machinery within endometriotic cells may contribute to the regulation of certain key genes involved in the differentiation process, leading to progesterone resistance. These epigenetic changes involve DNA and histone methylation and acetylation, as well as modification of coregulators such as activators, repressors, enhancers, miRs, and other non-coding RNA (36). Wu and colleagues first

reported the hypermethylation and silencing of the homeobox A10 (*HOXA10)* promoter in endometrial cells from women with endometriosis compared to controls (37). *HOXA10* is an integral homeobox gene family member involved in uterine development and function. *HOXA10* expression is normally increased in the mid-secretory endometrium under progesterone regulation. However, in eutopic endometrium of patients with endometriosis, *HOXA10* fails to increase its expression after ovulation. This is in part due to *HOXA10* promoter hypermethylation and gene-silencing (37). Furthermore, endometrial cells derived from women with endometriosis exhibit hypermethylation of the PR-B promoter resulting in decreased expression of the receptor protein (38). As previously mentioned, a decrease in PR-B expression and activity at the cellular level is indicative of a progesterone resistance state (1). Indeed, knockout studies have shown that the absence of PR-B in immortalized endometrial stromal cells yields unchecked proliferation aiding in implant establishment and maintenance (39).

Further evidence that the epigenetic environment is altered in endometriosis is exhibited by over-expression of the DNA methyltransferases that catalyze DNA methylation (e.g, DNMTs 1, 3A, and 3B). Wu and colleagues reported that DNMT1, 3a and 3b are over-expressed in ectopic endometrium from patients with disease compared to eutopic endometrium, rendering ectopic endometrium susceptible to hypermethylation of key transcription factors. Other evidence for epigenetic modifications includes the transcription factor steroidogenic factor - 1(SF-1), where reduced methylation in the gene promoter of endometriotic cells leads to increased SF-1 expression in ectopic implants and accounts for the high local production of aromatase. Furthermore, the ER-β promoter is hypomethylated in endometriotic implants, which may facilitate estrogen signaling and unchecked proliferation of cells in these implants (40). Conversely, hypermethylation may be a putative cause of significantly decreased expression of the motility-suppressing cell adhesion protein E-cadherin in endometriotic cells. Treatment with the histone deacetylase inhibitor, tricostatin A, restores E-cadherin expression and attenuates invasiveness of endometriotic cells (41).

MicroRNAs

Altered miR expression is another mechanism of epigenetic regulation in endometriosis. miRs are non-coding RNA fragments that function to inhibit protein expression by hybridizing with and causing degradation of mRNAs (42). Exploring this novel concept of gene regulation, Burney and colleagues conducted array-based miR profiling in early

secretory endometrium of patients with and without endometriosis (43) and found that miR-9 was significantly downregulated in patients with endometriosis compared to controls. One predicted target of miR-9 is *BCL2*, a gene encoding the anti-apoptotic protein known to be over-expressed in endometrium of patients with endometriosis. Similarly, three members of the miR-34 family, hypothesized to play a role in p53-dependent suppression of proliferation, were also down-regulated in the early secretory endometrium of patients with disease. In one study, miR-196a was found to be overexpressed in eutopic endometrium of patients with endometriosis, while its target, PR-B was found to be significantly decreased (44). Another miR implicated in progesterone resistance is the increased expression of miR-29c in endometriotic tissue. Its target is FK506 binding protein 4 (FKBP4) – a known progesterone regulated protein responsible for decidualization (45). The exact mechanisms by which miR expression is altered remain to be elucidated, but it has been proposed that reduced miR expression is the result of altered methylation of miR gene promoters, as treatment with demethylation agents restores normal expression (46). Taken together, these findings suggest differential regulation of miRs may serve as a conduit to impaired progesterone action in endometriosis.

Retinoid resistance

Like steroid hormones, retinoids are diet-derived lipids that serve as key paracrine mediators of progesterone action in the endometrium. Altered retinoid production and action may be a deleterious consequence of progesterone resistance in endometriosis (47). Systemic circulating progesterone is delivered to the endometrial stroma, and in turn, stromal cells utilize paracrine signaling to elicit epithelial genotypic and phenotypic differentiation in response to progesterone. One key paracrine mediator of such action is retinoic acid (RA). For instance, RA has been shown to stimulate the epithelial enzyme 17βHSD2 that catalyzes the oxidation of estradiol to less potent estrone. New evidence is emerging that RA action may be impaired in patients with endometriosis (47). Expression of vitamin A receptor (STRA6) and cellular retinol binding protein 1 (CRBP1), responsible for the uptake and transport of RA, respectively, is significantly reduced in stromal cells of patients with endometriosis compared to controls. Similarly, expression of retinaldehyde dehydrogenase 1 A2 (ALDH1A2), the enzyme responsible for conversion of retinol to RA, is also decreased. Intracellular shuttling of RA to the nucleus is impaired due to decreased expression of cellular retinoic acid binding protein 2

(CRABP2) and fatty acid-binding protein, epidermal (FABP5) which are responsible for delivery to retinoid receptors RARα/RXRα (retinoic acid and retinoind X receptors α), and PPARβ/δ (peroxisome proliferator activated receptors β and δ), respectively. Levels of CRABP2 are drastically reduced while levels of FABP5 are minimally reduced, leading to preferential shuttling of RA to PPARβ/δ. Expression of all known RA receptors has been found to be decreased in endometriotic tissue (47). Conversely, enzymes responsible for the catabolism of RA, such as the RA-metabolizing member B1 enzyme of the *P450* superfamily, are significantly increased.

Impaired action of progesterone is likely responsible for altered RA functionality in stromal cells of endometriotic tissue. STRA6 and CRABP2 are activated by progesterone in stromal cells, and knockout studies have shown that decreased expression leads to an anti-apoptotic, proliferative phenotype. Additionally, due to progesterone resistance, there is preferential shuttling of RA to PPARβ/δ, as previously mentioned, which may promote cell-survival (48). Altogether, these findings illustrate that the inability of progesterone to upregulate RA action yields a proliferative state with minimal differentiation, thereby promoting endometriotic cell survival. Based on emerging evidence, RA and RA agonists may play a therapeutic role in treatment due to their potential for surmounting progesterone resistance in disease states. Indeed, treatment of endometriotic cells with fenretinide, a synthetic retinoid analogue, has been shown to increase apoptosis and decrease cellular proliferation (49). Further studies are needed to firmly establish the therapeutic role of RA in endometriosis, keeping in mind the teratogenic potential of this class of drugs.

Correction of progesterone resistance

Synthetic progestins, dienogest and alternative routes of administration

Norethindrone acetate has been successfully utilized in the treatment of endometriosis related pelvic pain (50) and estroprogestins, via their inhibition of ovulation and reduction in prostaglandin production, decreased dysmenorrhea, pelvic pain, bleeding irregularities and even postoperative endometrioma recurrence (51). But we have provided evidence that progestin treatment may be of limited efficacy in endometriosis due to a state of progesterone resistance. Indeed, while progestins have been utilized for therapy with some efficacy, pain-relief can be short-term and a subset of patients fails to respond entirely (52). As such, therapies that overcome progesterone resistance have been sought for some patients with endometriosis. Dienogest (17α-cyanomethyl-17β-hydroxyestra-4,9-dien-3-one) is efficacious

in the treatment of endometriosis, attributed in part by its ability to overcome attenuated progesterone responses (53). Dienogest (DNG) is structurally related to norethindrone but differs by having a cyanomethyl group instead of an ethinyl group at C-17 and by the addition of a C-9-C-10 double bond (54). DNG in doses of 2 mg/day inhibits ovulation and downregulates proinflammatory cytokines, including IL-6, IL-8, and monocyte chemoattractant protein-1 (55). Additionally, DNG has direct inhibitory effects on aromatase expression in endometrial cells (56). Double-blind clinical trials have shown 2 mg DNG to be superior to placebo in pelvic pain relief of patients with all stages of endometriosis (57) and to improve all aspects of symptomatic endometriosis burden including dysmenorrhea, and irregular bleeding (58). One study showed that DNG may overcome progesterone resistance by directly increasing the PR-B:PR-A RNA ratio in endometriotic cells and tissues (53). ER-β, which is increased in endometriotic tissue, is downregulated by DNG (53). As ER-β can repress estrogen-induced PR expression, decreasing ER-β with DNG may enhance progesterone sensitivity in patients with endometriosis.

Treatment with other high potency progestins may be of benefit in overcoming mild progesterone resistance by inducing decidualization in eutopic and ectopic endometrium. Oral and depot formulations of medroxyprogesterone acetate (MPA) and other progestins have proven efficacy in management of endometriosis related pain and may be advantageous to GnRH agonists with respect to side effects (59) and cost (60). Subcutaneous administration of depot medroxyprogesterone acetate (104 mg) has equal efficacy to intramuscular GnRH agonist (11.25 mg) administration in reducing pain symptoms in patients with mild to moderate endometriosis, with less hypoestrogenic symptoms than the latter (61). The levonorgestrel intrauterine system (LNG-IUS) also has proven benefits, reducing moderate to severe dysmenorrhea in approximately 88% of patients compared to placebo (62). Eutopic and ectopic endometrial tissue biopsies after 6 months of LNG-IUS placement show a reduced cell-proliferation index in addition to decreased expression of ERα and PR-A (63). Symptomatic improvement of chronic pelvic pain seems equally promising, with some studies noting similar pain profiles in patients treated with LNG-IUS compared to GnRH analogues (64). Side effect profiles, similarly, are better in patients with LNG-IUS compared to GnRH analogues.

Other Strategies

While extant interventions to effectively enhance progesterone responsiveness in endometriosis are limited, new therapeutic approaches targeting the underlying cellular and molecular basis of progesterone resistance may prove efficacious. One study recently reported promising results for fenretinide, a low toxicity retinoid administered to overcome the decreased RA signaling in patients with endometriosis (49). Fenretinide exposure to endometriotic cells increased expression of genes involved in RA uptake and action, including STRA6. Furthermore, in mice bearing xenografted human endometriosis tissue treated with fenretinide for 2 weeks, endometriotic lesion volume was decreased. Orally active, non-peptide GnRH antagonists are currently under development. These have the advantage of dose adjustment such that systemic estradiol concentrations are only modestly suppressed, mitigating some of the hypoestrogenic side-effects of pituitary suppression (65). Anti-inflammatory drugs have the potential to normalize the progesterone resistance characteristic of pro-inflammatory states. For instance, statins have proven capacity to decrease the proinflammatory mediators IL-1β, TNF-α, and C-reactive protein. This class of drugs also inhibits angiogenic factors, further assuaging endometriotic lesion establishment and disease burden (66). An alternative perspective, well developed in the endometrial carcinoma field, is that as a consequence of progesterone resistance, sensitization to estrogens is amplified (67). This provides a rationale for aromatase inhibition for the treatment of endometriosis-associated pain (68). We hope that our speculations here will encourage independent investigators and pharmaceutical innovators to open new vistas for treatment of this disorder.

Conclusions

Endometriosis is a disease with a complex etiology and pathophysiology. While retrograde menstruation occurs in a majority of menstruating women, only a subset of women develops endometriosis. Hence, while the seeding of endometrial implants in the peritoneal cavity appears to be a key feature of this disease, the establishment of endometriotic lesions requires additional factors that capacitate invasion and growth. The pathogenesis of this disease likely originates in the eutopic endometrium itself, with progesterone resistance and impaired decidualization of effluxed endometrium being root causes of establishment and maintenance of ectopic implants. While the exact mechanism of progesterone resistance is yet to be elucidated, aberrations in genetic and epigenetic regulation of PRs and their targets have been demonstrated. Inflammation and detrimental environmental factors also may be implicated in

disease genesis via alterations in progesterone response. Deciphering the various pathways through which progesterone action is impaired in the eutopic endometrium of women with endometriosis is a crucial goal for disease prevention and treatment and may lead to identification of novel biomarkers to assist with prediction of response to future therapeutics.

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