Multiple Beneficial Roles of Repressor of Estrogen Receptor Activity (REA) in Suppressing the Progression of Endometriosis

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Endometriosis is an estrogen-dependent, inflammation-driven gynecologic disorder in which endometrial tissue creates inflammatory lesions at extrauterine sites, leading to pelvic pain and impaired fertility. Although dysregulated estrogen receptor (ER) signaling has been implicated, understanding of this disease is incomplete and current therapies are of limited benefit. Using an immunocompetent syngeneic murine model, we used combinations of donor uterine tissue and/or recipient host mice with partial genetic deletion of the ER coregulator, repressor of ER activity (REA) (also known as prohibitin 2), to investigate roles of REA in the contributions of donor uterine tissue and host cell influences on endometriosis establishment and progression. Ectopic lesions derived from donor tissue with half the wild-type gene dosage of REA (REA\(^{-/-}\)) grown in REA\(^{-/-}\) hosts displayed enhanced proliferation, vascularization, and markedly increased neuron innervation and inflammatory responses, including elevated cytokine production, nuclear factor kappa B activation, cyclooxygenase-2 expression, and immune cell infiltration. Although lesion progression was greatest when REA was reduced in both donor tissue and host animals, other donor/host combinations indicated that distinct stimulatory inputs were derived from ectopic tissue (proliferative signals) and host cells (inflammatory signals). Importantly, depletion of REA in primary human endometriotic stromal cells led to elevated proliferation and expression of cell cycle regulators. Notably, REA was significantly lower in human endometriotic tissue versus normal human endometrium. Thus, REA modulates cross talk among multiple cell types in the uterine tissue and host background, serving as a brake on the estradiol-ER axis and restraining multiple aspects that contribute to the pathologic progression of endometriosis. (Endocrinology 157: 900–912, 2016)
Endometriosis is complex in that it is promoted by and dependent upon extensive cross talk among the numerous cell types that comprise the endometriotic lesions. These include endometrial cells from the uterus, likely from retrograde menstrual flow, plus immune cells that infiltrate into the lesions, and vascular endothelial cells, blood vessels and nerves that grow into and support lesion establishment, survival and progression. Host peritoneal cells into which the lesion embeds may also contribute by providing a favorable environment for lesion survival. Many of these multiple cell types express estrogen receptors (ERs) and ER coregulators (5–7) that can work together to enhance or restrain the estrogen signaling that promotes progression of the disease.

A hallmark of endometriosis is excessive estrogen signaling (8, 9). This is supported by increased local production of estrogen driven by the aromatase gene, cytochrome P450, family 19, subfamily A, polypeptide 1 (1, 20), as well as altered expression of ERα and ERβ (11, 12). Therefore, current hormonal treatments, including progestins, androgens, GnRH agonists, and aromatase inhibitors, focus on reducing systemic levels of estrogens. These treatments, however, are limited by side effects, incomplete effectiveness, and high rates of disease recurrence after treatment cessation (2). To interrogate the molecular events underlying the establishment and progression of the uterine tissue at ectopic sites, murine models have been widely used recently (13). Critical roles of the ERs in endometriosis pathogenesis are also supported by studies employing ER knockout (KO) mice (14) and preclinical analyses with ER subtype-selective ligands (15–17). Because ER coregulators are recruited to chromatin by steroid hormone nuclear receptors in a temporally and spatially specific manner for precise gene regulation, these components also emerge as contributors to and potential therapeutic targets for controlling the multiple hyperestrogenic stimulatory activities that drive endometriosis establishment and progression.

The ER coregulator, repressor of ER activity (REA) (also known as prohibitin 2) (18, 19), has been shown to serve as a brake on ER activity in estrogen target tissues such as the uterus (20, 21) and mammary gland (22) and to affect cell signaling pathways. Homozygous ablation of REA in the uterus led to infertility due to severely compromised uterine development and failure of implantation (21). However, uteri of heterozygous REA+/− mice, with half the normal wild-type (WT) level of REA, showed an accelerated and amplified decidualization process and subfertility, due to hyperresponsiveness to estrogen signaling (20, 21, 23). These studies, documenting REA to be a physiologic, protective factor against excessive estrogen-driven activity in uterine tissue, led us to hypothesize that REA might also play a critical role in modulating or moderating the establishment and progression of endometriosis.

To explore this hypothesis, we have used an immunocompetent murine model, in which estrogen hyperstimulation of proliferation and inflammatory signaling in ectopic lesions recapitulate the disease in humans (24). Our findings reported herein using donor tissue and host animals with reduced levels of REA highlight distinctive roles that this ER corepressor plays in the ectopic uterine tissue and host tissues: normal levels of REA in ectopic uterine tissue restrain estrogen-supported implant growth and vascularization, whereas normal REA levels in host tissues suppress inflammatory responses associated with lesion progression. Elevated proliferative activity of human endometriotic stromal cells upon loss of REA and our comparison of human endometriotic tissue from patients vs normal human endometrium, which revealed significantly lower REA in endometriotic lesions further support the clinical relevance of REA and the usefulness of observations from our animal model. The findings highlight REA as a protective restraint on the estradiol (E2)-ER-driven axis in endometriosis that acts as a corepressor of multiple aspects of the pathologic progression of this disease.

Materials and Methods

Animals and immunocompetent mouse model of endometriosis

All animals were maintained in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals, and all procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. C57BL/6 mice were purchased from Harlan Laboratories or The Jackson Laboratory (EGFP, stock number 006567). REA heterozygous (REA+/−) mice on the C57BL/6 background and their WT littermates were maintained and genotyped as described previously (20, 23).

Endometriotic-like lesions were surgically transplanted as described before (17, 24). Briefly, female WT, EGFP, or REA+/− mice (8–10 wk of age) served as either donor or recipient animals, or both. The uterine horns were removed from donor mice, opened longitudinally, cut into fragments using a 3-mm dermal biopsy punch (Miltex) and transplanted onto the peritoneal wall of recipient mice by suturing. In each experimental group, uterine tissue was collected from at least 6 donor mice and transplanted into 6 recipient mice. Ectopic lesion volume was calculated as before (17).

To examine roles of REA in E2-supported lesion establishment, ovariectomized recipient mice were implanted with a pellet of E2 (Innovative Research of America) sc and underwent ectopic tissue transplantation on the same day. The dosage of 0.125-mg E2/pellet was chosen as optimal based on our previous work (17). At the times indicated, both eutopic uterine tissue and ectopic endometriotic lesions were collected for further analysis.
To interrogate functions of REA during chronic lesion progression, uterine fragments from donor mice were transplanted on alternate sides of the peritoneal incision into intact female recipients without any hormonal administration. The ectopic tissues were collected at 2, 4, and 8 weeks after transplantation surgery for further analysis.

In order to minimize hormonal variation in cycling mice, all donor mice used above, as well as intact recipients, underwent tissue transplantation at the diestrous stage. In addition, recipient mice were killed at diestrus for collection of tissues as described before (17, 24).

**Primary human endometrial and endometriotic stromal cell cultures and small interfering RNA (siRNA) studies**

Our studies involving human eutopic endometrial biopsies, endometriotic lesion biopsies, and primary cell cultures were approved by the Institutional Review Boards of the University of Illinois, Emory University, and Wake Forest University School of Medicine. All protocols adhere to the regulations set forth for the protection of human subjects participating in clinical research, including the establishment of a data and safety monitoring plan.

Isolation and culture of primary human endometriotic stromal cells were conducted as described (23). Cells were cultured in DMEM/F-12 medium (Invitrogen) containing 5% charcoal-dextran-treated fetal bovine serum. For siRNA experiments, endometriotic stromal cells were transfected with REA siRNA (siREA) or GL3 luciferase control siRNA (siGL3) (Dharmacon) following the Silent-Fect kit protocol (Bio-Rad Laboratories) as before (23). After 24 hours of transfection, cells were exposed to 20-ng/mL TNFα (R&D Systems) and 10nM E2 (Sigma-Aldrich) for the times indicated.

**Histological analyses, immunohistochemistry (IHC), and immunofluorescence**

IHC and immunofluorescence were performed in cultured cells or paraffin-embedded mouse or human tissue sections as described (23). Primary antibodies used (Supplemental Table 2) were: REA (Millipore Co), GFP (Cell Signaling Technology), Ki67 (Bioss), platelet endothelial cell adhesion molecule (PECAM) (Abcam), IL-6 (Invitrogen), p65 (Cell Signaling Technology), CD3 (Abcam), F4/80 (Acris Antibodies), COX2 (Abcam), protein gene product 9.5 (PGP9.5) (Abcam), progesterone receptor (PGR) (DAKO), and phosphohistone-H3 (P-H3) (Millipore Co). The stain signal was quantified by monitoring the average numbers of positively stained cells to the total number of cells from 6 randomly chosen fields.

**RNA isolation and real-time PCR**

Total RNA was isolated from eutopic or ectopic tissues or primary cells using TRIzol Reagent (Life Technologies) to prepare cDNA (17, 23, 24). Real-time PCR was performed to quantify gene expression using specific human or mouse primers (Supplemental Table 1) and SYBR Green kits (Bio-Rad Laboratories). After analysis by the delta cycle threshold method, data were normalized to 36B4 gene expression (23).

**Statistical analysis**

Statistical analyses included paired or unpaired t test, one- or two-way ANOVA with Bonferroni’s multiple comparison test and used GraphPad Prism version 5.00 (GraphPad Software). Data are expressed as mean ± SD, and P < .05 was assigned as statistically significant.

**Results**

**Impact of partial depletion of REA on E2-supported endometriosis-like lesion establishment**

We investigated the role of REA in E2-supported lesion establishment using different combinations of donor uterine tissue and recipient host background in which the level of REA was WT (REA+/+) or reduced (REA+/-). We first compared in parallel donor uterine tissue with different levels of REA transplanted into the same recipient animal. (Because REA null uteri displayed a severely atrophic phenotype [21], REA heterozygous [REA+/-] uteri were chosen for comparison with WT donor tissue with the full complement of REA [REA+/+].) As shown in Figure 1, A and B, after 2 weeks of E2 supplementation, both WT and REA+/- donor (D) uterine fragments, surgically transplanted onto the peritoneal surface of ovariecotomized WT recipient (R) mice, were able to form endometriosis-like lesions (Figure 1B), suggesting a restraining role for REA present in ectopic endometriosis-like lesion growth.

Although the heterozygous REA+/- transcript and protein levels (~50%) were confirmed in REA+/- donor uterine tissue compared with WT uterine by qPCR (Figure 1C) and IHC (Figure 1D), it was noteworthy that D+/-/Rwt ectopic lesions contained about 70% of the WT level of REA mRNA (Figure 1C) and REA protein (Figure 1E), implying a contribution of REA from infiltrating host cells. Indeed, as shown in Figure 1F, the presence of REA in host cells that infiltrate the ectopic lesion was validated by cellular colocalization of REA and EGFP proteins in EGFP-transgenic host mice by dual immunofluorescence analysis when WT donor uterine tissue was transplanted into EGFP recipients, and these recipients were given 2 weeks of E2 treatment.

In order to evaluate the importance of host cell REA in ectopic lesion progression, we examined donor tissue growth and phenotypic properties in E2-treated REA+/- recipient mice (Figure 2A). Although D+/-/Rwt ectopic tissues formed larger lesions compared with D+/-/Rwt lesions (Figure 1B), WT lesions established in REA heterozygous recipients (D+/-/R+/-) displayed a growth rate similar to that of D+/-/Rwt lesions (Figure 2B), implying that the proliferative signal in

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lesions is affected largely by the REA content in the donor tissue.

Because the host environment and cells that infiltrate into the ectopic lesion from the host animal can contribute to inflammatory aspects of the disease (17), we next examined the expression of several cytokines known to be highly expressed in human endometriotic tissue (25–28) and regulated by E2 signaling in endometriosis (17).

Figure 1. Ectopic lesions with a reduced level of REA show enhanced growth in E2-treated WT recipient mice. A, Endometriosis-like lesions were established by transplanting donor (denoted by D) uterine fragments from REA WT (REA⁺/⁺, n = 6) and REA heterozygous (REA⁺/-, n = 6) mice of diestrous stage into ovariectomized (OVX) REA⁺/⁺ recipient (denoted by R) mice, as described in Materials and Methods. B, Growth of ectopic lesions was monitored and lesion volume was quantified after 2 weeks of E2 treatment, \( P < 0.05 \) (paired \( t \) test). C, qPCR analysis of REA mRNA in donor uterine tissue and ectopic lesions after 2 weeks of E2 treatment. Levels of mRNA are expressed relative to the transcript level in REA⁺/⁺ donor uterine tissue which is set at 1.0. Different letters indicate \( P < 0.05 \) by one-way ANOVA with Bonferroni's multiple comparison test. D, Donor uterine tissue from REA⁺/⁺ and REA⁺/- mice at diestrous stage was subjected to IHC staining for REA. GE, glandular epithelium; LE, luminal epithelium; S, stromal tissue. E, IHC staining of REA in ectopic lesions after 2 weeks of E2 treatment. IgG served as negative control. C, endometriotic cyst; E, epithelial tissue; S, stromal tissue. F, Dual immunofluorescence of REA and GFP in ectopic lesions derived from WT donor tissue transplanted into WT EGFP recipient mice treated with E2 for 2 weeks. D, donor; R, recipient.
shown in Figure 2D, when compared with $D^{wt-Rwt}$ lesions, levels of IL-6 ($IL6$), chemokine (C-C motif) ligand 2 ($Ccl2$) and $Ccl5$ in $D^{wt-R+/-}$ ectopic lesions were increased, whereas the expression of TNFα ($Tnfα$) remained unchanged.

**REA expressed in donor uterine tissue suppresses ectopic cell proliferation and vascularization**

To further investigate functions of donor tissue REA in lesion progression, we used a model in which both REA donor WT ($D^{wt-Rwt}$) and REA donor heterozygous ($D^{+/-}$) ectopic tissues were allowed to become established in the same WT intact recipient mice, and growth was monitored over 8 weeks. As seen in Figure 3A, $D^{+/-}$-REA ectopic lesions showed an enhanced growth rate after 2 weeks of lesion progression compared with that of $D^{wt}$-$R^{wt}$ lesions. Notably, elevated ectopic lesion cell proliferation was seen in 4-week $D^{+/-}$-REA ectopic lesions vs $D^{wt}$-$R^{wt}$ lesions by IHC analysis of the proliferation marker Ki67 (Figure 3, B and C). By contrast, Ki67 staining analysis of eutopic uterine WT REA⁺/⁻ or REA⁺/+ donor tissues indicated no difference in cell proliferation (Supplemental Figure 1A), further suggesting the specific roles of donor REA level in lesion progression. Immunofluorescence for the blood vessel marker, PECAM (Figure 3D), and quantification of PECAM-positive cells as an indicator of vascularization (Figure 3E) also documented that $D^{+/-}$-REA lesions were more highly vascularized compared with $D^{wt}$-$R^{wt}$ ectopic lesions, but remained at a sim-
ilar level in eutopic tissue (data not shown). By contrast, cytokine production was similar in WT and Dwt-Rwt lesions, where cytokine production in the ectopic lesion is principally determined by the host level of REA in infiltrating cells.

Host REA restrains inflammation and innervation of ectopic endometriotic lesions

Next, both WT and REA+/- mice were used as recipients to interrogate the impact of REA in the host tissue on endometriotic lesions that developed from REA WT uterine tissue. Consistent with our observations for cytokine expression in WT lesions from E2-treated heterozygous recipients (Dwt-R +/- ) (Figure 2C), higher Il6, Ccl2, Ccl5, and Tnfa mRNA levels were seen by qPCR in WT lesions established in ovary intact REA+/- recipients (Dwt-R +/- ) and monitored over 8 weeks of progression (Figure 4A). Immunostaining also demonstrated a greatly increased level of IL6 protein and p65 protein in Dwt-R +/- lesions vs Dwt-Rwt lesions (Figure 4, B and C). The greater than 2-fold increase in the number of nuclear p65-positive cells in Dwt-R +/- ectopic lesions supports stronger nuclear factor kappa B activity in these lesions (Figure 4C). Immunofluorescence for the T-cell marker CD3 and macrophage marker F4/80 also revealed more immune cells in the Dwt-R +/- lesions (Figure 4D). COX2 protein, another hallmark of endometriotic lesion-associated inflammation (2), was also present at a higher level in Dwt-R +/- vs Dwt-Rwt lesions (Figure 4E). Also notable was the increased innervation of Dwt-R +/- lesions, observed by staining of the pan neuron marker, PGP9.5 (Figure 4E). Taken together, these data support suppressive roles of REA in host cells and tissues in multiple inflammatory responses and in lesion innervation that accompanies lesion progression. Notably, however, Dwt-R +/- ectopic tissues showed similar lesion growth rate (Figure 4F) and cell proliferation activity indicated by Ki67 staining (Supplemental Figure 1B) to that of Dwt-Rwt lesions, which is distinctly different from what was observed in Dwt-R +/- vs Dwt-Rwt lesions. These findings highlight the importance of REA status in donor uterine tissue on proliferative drive, in addition to distinctive contributions from the host environment in the inflammatory aspects of endometriosis.

Figure 3. Impact of REA level in donor uterine tissue on lesion progression. Donor uterine tissue from REA WT (REA+/-, n = 6) and REA heterozygous (REA +/-, n = 6) mice were transplanted into intact WT recipient mice (n = 6) and were followed over 8 weeks for formation of Dwt-Rwt and Dwt-R +/- lesions, respectively. Both donor and recipient mice underwent transplantation surgery at the diestrous stage. A, Growth of Dwt-Rwt and Dwt-R +/- lesion volume over time was quantified as shown. *, P < .05 (two-way ANOVA with Bonferroni’s multiple comparison test). B, IHC staining for Ki67 in Dwt-Rwt and Dwt-R +/- lesions after 4 weeks of progression in intact WT recipients. C, Quantification of Ki67 staining signals in lesions. *, P < .05 (paired t test). D, PECAM staining of vasculature in ectopic lesions at 4 weeks of progression in intact WT recipients. E, Quantification of PECAM-positive cells at 4 weeks. *, P < .05 (paired t test). BV, blood vessel; C, endometriotic cyst; E, epithelial tissue; S, stromal tissue. F, Il6, Ccl2, Ccl5, and Tnfa mRNA levels in Dwt-Rwt and Dwt-R +/- ectopic tissues were analyzed by qPCR at 4 weeks of lesion progression. Transcript levels are expressed relative to the transcript level in Dwt-Rwt lesions which is set at 1.0. No significant difference was detected.
Lesion growth and inflammation are most increased when REA level is reduced in both donor and recipient host tissues.

To further understand the actions of REA in the cross talk between donor and host tissues, REA<sup>−/−</sup> donor uterine tissue was transplanted into heterozygous REA host mice (D<sup>wt-R<sup>−/−</sup></sup>) and lesions were collected after 8 weeks. Quantification of lesion volume (Figure 5A) showed that the reduced gene dosage of REA in both donor and host tissues (D<sup>wt-R<sup>−/−</sup></sup>) resulted in the greatest...
Figure 5. Impact of reduced REA levels in both donor uterine tissue and host animals on lesion progression. Donor tissues from REA+/− mice at diestrous stage (n = 6) were transplanted into intact REA+/− recipients (n = 6) to form D+/−-R+/− lesions. At 8 weeks after transplantation, quantification of (A) lesion volume and (B) cytokine mRNA levels by qPCR were monitored in Dwt-Rwt, D+/−-R+, D+/−-R−, and D+/−-R+/− lesions. Donors and recipients were intact animals chosen from the diestrous stage. Different letters indicate P < .05 by one-way ANOVA with Bonferroni’s multiple comparison test.

REA regulates proliferation of human endometriotic stromal cells

To examine the functional significance of REA in human endometriotic cells from patient samples, we employed siRNA knockdown of REA in primary human endometriotic stromal cells cultured in vitro. Treatment with siREA resulted in greatly reduced levels of REA mRNA (Figure 6A) and protein (Supplemental Figure 3). When cells were exposed to TNFα and E2 to mimic the in vivo hyperestrogenic and inflammatory microenvironment characteristic of endometriosis (29, 30), the mRNA levels of several key cell cycle regulators, such as cyclin-dependent kinase 2 (CDK2), cyclin B2 (CCNB2), cyclin D2 (CCND2), and minichromosome maintenance complex 2, were more markedly elevated in siREA treated than in control siGL3-treated human endometriotic stromal cells, especially in cells treated with E2 + TNFα (Figure 6A). By contrast, the level of ERα was not changed (Figure 6A). Increased cell proliferation was evident from immunofluorescence assays of Ki67, P-H3, and by quantitation of cell numbers in cells with knockdown of REA (Figure 6B). Thus, REA normally acts as a suppressor of human endometriotic cell proliferation, reflected by enhanced expression of key cell cycle regulators and proliferation markers when REA was reduced in the cells.
REA is lower in human endometriotic tissue vs normal human endometrium

As shown in Figure 7, we compared by IHC the presence of REA protein in normal eutopic endometrium (n = 4 women, ages 25–44) and in ectopic endometriosis samples from women with the disease (n = 12 patients, ages 22–50). Quantitation of REA in tissue sections (3 tissue blocks per patient and 6 fields quantitated per section from each block) revealed that REA was significantly lower in endometriosis samples compared with normal eutopic endometrium.
Discussion

Estrogen and inflammatory signaling, which are controlled by nuclear receptors and their coregulators, are essential for the survival of endometriotic tissue and for disease progression (31, 32). Endometriotic tissue, like normal uterine tissue, is reliant on estrogen, but endometriosis is unique in that the endocrine milieu and hormone receptor status of the endometriotic lesions are very different from those in normal reproductive tissues. In particular, estrogen production and ER regulation are altered in endometriotic lesions. The ectopic tissue overexpresses aromatase and COX2 (2, 33), thereby causing continuous local production of estrogens and prostaglandins. Also endometriotic lesions have increased levels and increased activity of ERs which elicit a state of hyperstimulation (11, 12, 34) that drives progression of the disease. Coregulators partner with ERs to control receptor activity and, in this study, we have found that the corepressor, REA, functions as a restraint on ER to suppress the estrogen-stimulated proliferative drive of endometriotic lesions. Thus, when REA was reduced, it exacerbated and promoted pathologic progression of the disease.

Our observations highlight and support the existence of extensive cross talk among various cell types that collaborate to support the growth and phenotypic properties of the endometriotic lesions; these include the ectopic uterine endometrial cells and supporting cells from the immune, nervous and vascular systems (17, 35) that are found infiltrating the ectopic lesions as they develop (1, 36). Our studies using host EGFP transgenic mice demonstrated infiltration of host cells into the ectopic lesion and an enhanced macrophage-monoocyte complement in heterozygous REA+/− host animals. Our findings indicate that REA modulates this cross talk between donor uterine-derived cells and infiltrating host cells, and that reduction of the REA level in both donor and host tissues most greatly accelerates the growth and inflammatory signaling in endometriotic lesions.

Notably, as shown in the model in Figure 8, our findings suggest that the growth and inflammatory signals that contribute to endometriotic lesion progression originate principally from distinct tissue loci, with stimulatory inputs from the ectopic uterine tissue primarily responsible for control of lesion proliferation and vascularization, and host cells and tissues primarily responsible for control of inflammation and neurogenesis in lesions. Our use of different combinations of donor tissue and host backgrounds, that allow modulation of REA gene dosage in each, enabled us to specify distinct stimulatory inputs from the ectopic uterine donor and host cells. However, the donor/recipient experimental observations also have revealed that the donor and recipient tissues impact each other in ways that influence the progression of endometriosis, because lesion growth and inflammatory signaling were greatest when both the uterine donor tissue and the recipient host mice were heterozygous for REA.

Of note, our studies in primary human endometrial stromal cells in which REA levels were experimentally reduced revealed that REA normally restrains proliferation so that its depletion resulted in elevated proliferative activity and enhanced expression of cell cycle regulators. Furthermore, in clinical specimens, REA was found to be significantly lower in human endometriotic lesions from patients compared with normal endometrium. The find-
ings with clinical samples support observations made in our preclinical mouse endometriosis model and suggest a critical role for REA in the pathologic progression of endometriosis.

Previous studies have documented that REA represses ER signaling (21) and exerts modulatory roles on pathways controlling cell survival and metabolism consistent with its name also as prohibitin 2 (18, 19, 37). REA has been established as a key ER corepressor in the mammary gland and female reproductive tract, as well as in breast cancer cells (20–23). In the current study, we have highlighted the pleiotropic ability of REA to suppress lesion progression by modulating multiple aspects of estrogen-mediated signaling in endometriosis. The findings provide evidence that this coregulator acts as a restraint on ER activities, repressing ER signaling that contributes to the pathologic molecular milieu in endometriotic lesions. Some proteins that function as coactivators of ER have also been shown to impact endometriosis. For example, the coactivator steroid receptor coactivator-1 is cleaved by TNFα-activated matrix metalloproteinase 9 into a cytoplasmic 70-kDa shortened isoform, which notably prevents TNFα-mediated apoptosis in ectopic endometriotic cells (38). Also of interest, endometrial deficiency of the transcription factor Krüppel-like factor 9, which acts as a regulator of ERα signaling, promoted endometriotic lesion establishment and affected notch-, hedgehog-, and steroid receptor-regulated pathways (39). Thus, both co-activators and corepressors appear to regulate key aspects in the pathogenesis of endometriosis.

Endometriosis is associated with chronic inflammation (1–4) and in recent results from murine models, Burns et al reported that compared with WT lesions transplanted into WT hosts, WT lesions were proliferative in ERα KO recipient mice but showed decreased inflammatory responses upon E2 treatment (14). Consistent with this, the novel ER ligand, oxabicycloheptene sulfonate, with preferential affinity for ERα, lost its suppressive effects in WT ectopic lesion-associated inflammatory responses in ERαKO recipients (17) or in WT recipient mice depleted of macrophages with clodronate liposomes (17), suggesting critical roles of ERα and host myeloid responses.
In the current work, we have used an immune-intact syngeneic murine model, in which the impacts of donor and host REA could be clearly distinguished and compared. We found that E2-supported chronic inflammatory responses in intact animals that mimic clinical findings. These include cytokine production, nuclear factor kappa B activation, Cox2 expression and immune cell infiltration, all of which were elevated upon partial loss of host but not donor REA. By contrast, donor uterine tissue REA level was most important in the control of ectopic lesion proliferation and vascularization. These findings underline the cellular and functional complexity of endometriosis lesions and support separate contributions of the donor tissue and the host environment in the proliferative and inflammatory aspects of endometriosis driven by the estrogen-ER axis.

Lesion innervation is thought to be involved in endometriosis-associated pain (40–42), and we showed previously that treatment with dual antiestrogenic and anti-inflammatory compounds suppressed the innervation of murine endometriotic-like lesions (17). Interestingly, neuroangiogenesis, a critical process driving the disease, has been shown to be regulated by E2 signaling by Greaves et al (43). Moreover, E2 is not only able to stimulate macrophage infiltration into ectopic lesions but also to activate interactions of macrophages and nerves, and thus may exacerbate endometriosis-associated pain (44). The current findings of reduced REA increasing lesion inflammatory signaling and nerve innervation suggest that by impacting E2-ER signaling, host-derived REA might serve as a potential regulator of pain in endometriosis.

Current medical management of endometriosis patients, which is primarily focused on suppressing E2 production (2), has not proven to be fully satisfactory. ERs, which are known to be essential and dysregulated in the pathogenesis of endometriosis, and their coregulators emerge as promising therapeutic targets. For example, our novel ER ligands, oxabicycloheptene sulfonate and chloroindazole, displayed dual suppression of estrogenic and inflammatory activities and were effective in preventing the establishment and progression of endometriotic lesions in mice (17). The selective ER modulators, bazedoxifene (15) and ERB-041 (16), have also been shown to suppress endometriotic lesion growth. Because REA/prohibitin 2 suppresses the proliferation of human endometriotic stromal cells and endometriosis-like lesion progression in the preclinical mouse model, and is reduced in human endometriotic tissue compared with its level in normal human endometrium, it appears that maintenance of adequate levels of REA may be important in preventing the development of this disease. The clinical relevance of our study is also highlighted by our observation that neuron innervation, which may be involved in the chronic pelvic pain of endometriosis (40–42), was also suppressed by host REA. Therefore, our findings provide new insights into critical roles of coregulators in endometriosis, and imply that novel therapeutic approaches based on modulation of such coregulators might hold future potential for improving medical care of women with this challenging disease.

Acknowledgments

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