Endometriosis expresses a molecular pattern consistent with decreased retinoid uptake, metabolism and action[†]

Mary Ellen Pavone^{1,2,*}, Matthew Dyson¹, Scott Reirstad¹, Elizabeth Pearson¹, Hiroshi Ishikawa¹, You Hong Cheng¹, and Serdar E. Bulun^{1,2}

¹Department of Obstetrics and Gynecology, Division of Reproductive Biology, Feinberg School of Medicine at Northwestern University, 303 Superior Street, Suite 4-123, Chicago, IL 60611, USA ²Division of Reproductive Endocrinology and Infertility, Feinberg School of Medicine at Northwestern University, 303 Superior Street, Suite 4-123, Chicago, IL 60611, USA

*Correspondence address. Tel: +1-312-503-0520; Fax: +1-312-503-0095; E-mail: mpavone@nmff.org

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BACKGROUND: Retinoic acid (RA) regulates key biological processes, including differentiation, apoptosis and cell survival. RA mediates induction of 17 beta-hydroxysteroid dehydrogenase type 2 mRNA, catalyzing the conversion of estradiol to estrone, in endometrium but not endometriosis because of a defect in endometriotic stromal cells. This defect may involve both the uptake and metabolism of RA. In this study, we analyze the expression of genes involved in RA signaling in normal endometrium and endometriosis.

METHODS: Tissue and stromal cells from ovarian endometriomas and eutopic endometrium from disease-free women were collected. Real-time reverse transcription-polymerase chain reaction was used to measure mRNA levels. Western blotting was used to evaluate protein expression.

RESULTS: We found that endometriotic tissue and stromal cells demonstrated significantly decreased mRNA expression of the major genes involved in RA signaling, including STRA6, CRBP1, ALDH1A2, CRABP2 and FABP5. We found increased levels of CYP26B1, responsible for RA metabolism. Nuclear extracts showed that RAR α , RXR α and PPAR β/δ were underexpressed in both tissues and stromal cells from endometriotic tissue. Differences in protein levels were confirmed by western blotting.

CONCLUSIONS: Endometriosis is characterized by a gene expression pattern suggesting a decrease in uptake and metabolism of RA. Because RA is integral in regulating key biological processes involved in cell survival, this alteration could partially explain the resistance to apoptosis found in endometriosis.

Key words: retinoid / retinoic acid / endometrium / endometriosis / apoptosis

Introduction

Endometriosis is often described as a disease state characterized by inappropriately reduced apoptosis (Beliard *et al.*, 2004; Wu *et al.*, 2008; Nasu *et al.*, 2009). There is a growing trend in the literature suggesting altered retinoid metabolism in endometriosis (Beliard *et al.*, 2004; Cheng *et al.*, 2006, 2007; Burney *et al.*, 2007; Bulun, 2009; Delvoux *et al.*, 2009; Bulun *et al.*, 2010; Dassen *et al.*, 2010; Pavone *et al.*, 2010). Our laboratory has shown that in normal endometrium, progesterone induces stromal cells to secrete paracrine

factors, which then stimulate neighboring epithelial cells to express mRNA for the enzyme 17 beta-hydroxysteroid dehydrogenase type 2 (HSD17B2). HSD17B2 catalyzes the conversion of E2 to estrone (E1); thus, progesterone signaling through the endometrial stroma regulates estrogen metabolism in the endometrium. In endometriotic tissue, progesterone does not induce epithelial HSD17B2 mRNA expression due to a defect in stromal cells. The end result is deficient metabolism of E2 in endometriotic tissues giving rise to high local concentrations of E2 (Cheng *et al.*, 2006, 2007; Bulun, 2009; Pavone *et al.*, 2010). We have shown that retinoic acid (RA) is one of the paracrine

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factors mediating the progesterone-dependent induction of HSD17B2, and we hypothesize that defective retinoid metabolism in endometriotic stromal cells contributes to reduced E2 metabolism and increased cell survival in endometriosis (Nobel et al., 1997; Bulun et al., 2006; Cheng et al., 2006, 2007; Bulun, 2009; Bulun et al., 2010; Pavone et al., 2010).

RA controls the proliferation and differentiation of numerous cell types, and is indispensable for the development and maintenance of many tissues, including the eye, brain, ovary and heart. RA is derived solely from dietary sources of vitamin A, which are taken up in the intestine and packaged as retinyl esters in hepatic stellate cells. From the liver, the esters are secreted into circulation as retinol bound to its specific plasma transport protein, retinol binding protein (RBP). In a pioneering 2007 paper, Kawaguchi et al. (2007) found Stimulated by RA 6 (STRA6) to be an essential cell surface receptor for RBP, and necessary for retinol uptake into cells. We previously demonstrated that STRA6 is the main regulator of retinol uptake in the endometrium and that the decreased expression of this gene in endometriosis can contribute to decreased HSD17B2 mRNA expression (Pavone et al., 2010).

Once retinol is brought into the cell by STRA6, it is sequentially oxidized, first by alcohol or short-chain dehydrogenases, and finally by one of the aldehyde dehydrogenases (ALDH) to generate the more biologically active RA. RA may then serve in either an intracrine or paracrine role. Within the cell, RA can be shuttled from the cytoplasm to the nucleus by carrier proteins, including cellular RA binding protein 2 (CRABP2) and fatty acid binding protein 5 (FABP5). Through these mechanisms, RA is targeted to specific nuclear RA receptors present within that cell to regulate gene expression. Alternatively, RA can function in a paracrine manner, being secreted to nearby cells where it may also regulate gene expression. In either case, excess RA may be oxidized to less active but more polar metabolites by the CYP26 family of enzymes, which permit the elimination of retinoids from the cell. The balance between the autocrine and paracrine activity of RA in a cell depends on its expression pattern of the genes that metabolize, traffic and respond to these retinoids (Napoli, 1999; Deng et al., 2003).

The biological actions of RA are mediated by several families of transcription factors recognizing RA as a ligand. The best characterized of these transcription factors are the RA receptors [retinoic acid receptors (RAR) α , β , γ], which bind to retinoid X receptor (RXR) to form heterodimers. These heterodimers bind to regulatory elements in target genes and modulate transcription, and it is through these transcription factors that RA can trigger differentiation, cell cycle arrest and apoptosis. Recently, Schug et al. (2007, 2008) found that RA binds to PPAR β/δ , another nuclear receptor that can heterodimerize with RXR. Notably, the binding of RA to PPAR β/δ induces the expression of pro-survival genes. Moreover, the partitioning of RA between RAR α and PPAR β/δ is regulated by the intracellular lipid binding proteins CRABP2 and FABP5. It was demonstrated that RA functions through RAR α to promote apoptosis in cells that express a high CRABP2:FABP5 ratio, and through PPAR β/δ to promote survival in cells that contain lower levels of CRABP2 (Schug et al., 2007, 2008). We recently found a decreased CRABP2:FABP5 ratio in endometriotic stromal cells, which may contribute to their anti-apoptotic phenotype (Pavone et al., 2010).

STRA6 and CRABP2 expression are regulated by the progesterone receptor in the endometrium, but all three of these genes are reduced in endometriosis (Bulun et al., 2006; Cheng et al., 2006, 2007; Bulun, 2009; Pavone et al., 2010). Given that this profile correlates with increases in cell survival, we suspect that the anti-apoptotic phenotype characteristic of endometriosis may be, in part, related to an altered CRABP2:FABP5 ratio (Schug et al., 2007, 2008; Pavone et al., 2010). However, micro-array results suggest that there is a significant variation in both retinoid uptake and metabolism in endometriotic tissue (Aghajanova et al., 2011). We believe that the perturbation in both uptake and metabolism is significant and sought to explore it as an additional mechanism contributing to decreased apoptosis in endometriotic tissues. We hypothesize that the resistance to apoptosis noted in endometriosis may, in part, occur because of a defect in both retinoid uptake into stromal cells as well as retinoid metabolism. To verify this hypothesis, we investigated genes involved in RA signaling in endometrium and endometriosis, first concentrating on the genes suggested by the micro-arrays, and then examining other genes involved in this pathway.

Materials and Methods

Tissue acquisition

Eutopic endometrium ($n = 10$) from women undergoing hysterectomy for benign indications other than endometriosis and ectopic endometrium from the cyst walls of ovarian endometriomas ($n = 10$) were obtained immediately following surgery. None of the patients had received preoperative hormonal therapy, and all samples were histologically confirmed. All samples were from the proliferative stage of the menstrual cycle from premenopausal women. The phase of the menstrual cycle was determined by both preoperative history and histologic evaluation of the endometrium. The average age of the patients was 40.1 ± 6.1 year for endometrium and 36.1 ± 3.1 for endometriosis, and this difference was not statistically significant. Written informed consent for obtaining the tissue was obtained prior to surgery. This consent was approved by the Northwestern Institutional Review Board for Human Research. Each sample was divided and used for mRNA and protein tissue extraction as well as stromal and epithelial cell isolation for mRNA and protein.

Tissue freezing

Immediately following surgery, a portion of tissue was snap-frozen in liquid nitrogen. This tissue was used for both RNA and protein extraction.

Stromal and epithelial cell isolation

With the remainder of the tissue, stromal and epithelial cells were isolated using a previously described protocol, with minor modifications (Ryan et al., 1994; Noble et al., 1997). Briefly, tissues were minced, digested with collagenase (Sigma, St Louis, MO, USA) and DNase (Sigma) at 37°C for 30 min, then with collagenase, DNase, pronase (Sigma) and hyaluronidase (Sigma) for an additional 20 min. Epithelial and stromal cells were separated by filtration through 70 and 20 μ m sieves. These cells were then either immediately rinsed with phosphate-buffered saline (PBS) and placed in TRIzol reagent (Sigma) for mRNA isolation, or resuspended in Dulbecco modified Eagle medium (DMEM)/F12 1:1 (GIBCO/BRL, Grand Island, NY, USA) containing 10% fetal bovine serum and grown in a humidified atmosphere with 5% CO₂ at 37°C. Purity of the stromal and epithelial cells was verified microscopically.

RNA extraction and quantitative real-time RT-PCR

Total RNA from either tissue or stromal cells was isolated using TRIzol reagent following the manufacturer's protocol. One microgram of RNA was then used to make cDNA using q-script cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA). Real-time quantitative PCR was performed with the ABI 7900 Sequence Detection and the ABI Power Syber Green gene expression systems (Applied Biosystems, Foster City, CA, USA). Messenger RNA levels for STRA6, CRBP1, ALDH1A2, CRABP2, FABP5, CYP26B1, RAR α , RXR α , PPAR β/δ and 18S were quantified. 18S was used for normalization. Relative quantification of mRNA species was done using the comparative threshold (CT) cycles method. For each sample, we normalized the gene CT value using the formula: $\Delta CT = CT_{\text{gene}} - CT_{18S}$. For relative expression levels, the following formula was used: $\Delta\Delta CT = \Delta CT_{\text{sample}} - \Delta CT_{\text{calibrator}}$. This value was then used to plot the gene expression employing the formula $2^{-\Delta\Delta CT}$.

For CRBP1, ALDH1A2, CRABP2, FABP5, RAR α , RXR α , PPAR β/δ and CYP26B1, commercially available primers were used (QIAGEN, Valencia, CA, USA). For STRA6 mRNA, the following primers were used: forward, 5'-TGTTGTCCTGCTTACTCACCTTC-3' reverse, 5'-GAAGCTCATCC AACAGAATATGG-3'; for 18S: forward, 5'-AGGAATTCCTCCAGTAA GTGCG-3' reverse, 5'-GCCTCACTAAACCATCCAA-3'.

Nuclear extract isolation

Stromal cell nuclear extracts were prepared using the NE-PER nuclear extraction kit (Pierce, Rockford, IL, USA) following manufacturer's instruction. These samples were used for immunoblotting as described in the following section.

Immunoblotting

Frozen tissue was crushed and then suspended in tissue protein extraction reagent (Pierce). Stromal cells were washed with ice-cold PBS and suspended in mammalian protein extraction reagent (Pierce). Lysates were cleared by centrifugation at 14 000g for 10 min. Equal amounts of protein (40 μ g for tissue, 20 μ g for stromal cells, 10 μ g for nuclear extracts) were resolved on 4–20% Ready Gel Precast Gels (Bio-Rad Laboratories, Hercules, CA, USA), for 90 min at 60 mAmp and transferred to a 0.45 mm pore nitrocellulose membranes at 150 mAmps for 2 h. The membranes were blotted for a minimum of 1 h in a solution of 1% dried milk in Tris-buffered saline at room temperature and incubated with the primary antibodies at 4°C overnight (STRA6 antibody graciously donated; CRBP1, ALDH1A2, CRABP2, FABP5, RAR α , RXR α , CYP26B1 and PPAR β/δ antibodies from Abcam, Cambridge, MA, USA). The membranes were then washed and incubated with the appropriate secondary antibodies for 1 h. Beta-actin or TATA-binding protein were used as loading controls. Detection was performed using a Supersignal West Femto Maximum Sensitivity Substrate System (Pierce).

Statistical analysis

Statistical differences between sample means were determined using the Wilcoxon signed rank test for non-parametric statistical evaluation. Values were expressed as mean $\pm$ SEM, with a $P < 0.05$ considered to be statistically significant. For all experiments, mRNA expression was measured, normalized to 18S and expressed as relative mRNA levels found in endometrial tissue as a multiple of that found in the endometriotic tissue. Western blots were quantified using ImageJ software (NIH.gov). These data are represented as band intensity of endometrium and endometriosis relative to actin.

Results

STRA6 is differentially expressed in endometrium versus endometriosis

Microarray suggested a differential expression of STRA6, the gene that we previously demonstrated to be integral in retinoid uptake in endometrial stromal cells. Therefore, we first investigated whether STRA6 was differentially expressed in endometrium and endometriotic tissues. We previously demonstrated that STRA6 levels were significantly higher in endometrial stromal cells compared with endometriotic stromal cells; so we hypothesized that tissue levels would confirm these results. To verify this, tissue levels of STRA6 mRNA expression were measured. As shown in Fig. 1, STRA6 was differentially expressed in endometrium compared with endometriotic tissue, with higher levels present in endometrium. Western blots confirmed this result.

STRA6 is expressed predominantly in endometrial stromal cells

We then investigated whether STRA6 was preferentially expressed in epithelial or stromal cells. Our previous work suggested that retinol uptake predominantly took place in stromal cells. To verify this, we analyzed STRA6 mRNA expression in epithelial and stromal cells. As shown in Fig. 2A, mean STRA6 levels were significantly higher in endometrial stromal cells compared with those in epithelial cells, indicating that retinol uptake is more likely to occur in stromal cells.

CRBP1 is differentially expressed in endometrial versus endometriotic tissue and stromal cells

To determine whether CRBP1 was differentially expressed in endometrium and endometriosis, tissue and stromal mRNA and protein expression of this gene were examined. As shown in Figs 1 and 2, this gene was differentially expressed when tissue and stromal cells were examined, with both mRNA and protein levels being significantly higher in endometrial tissues and stromal cells compared with those in endometriotic tissues and stromal cells.

ALDH1A2 is differentially expressed in endometrial versus endometriotic tissue and stromal cells

To determine whether ALDH1A2 was differentially expressed in endometrium and endometriosis, tissue and stromal mRNA and protein expression of this gene were examined. As shown in Figs 1 and 2, ALDH1A2 mRNA and protein expression were significantly higher in endometrial tissue and endometrial stromal cells compared with endometriotic tissue or stromal cells, suggesting that there is reduced RA production in endometriotic tissues and stromal cells when compared with those in endometrial tissue and stromal cells.

CRABP2 is differentially expressed in endometrial versus endometriotic tissue and stromal cells

We next explored the expression of CRABP2. We have previously shown that this gene is differentially expressed in endometrial and endometriotic stromal cells, with higher levels being present in endometrial stromal cells. To confirm that CRABP2 was differentially expressed in endometrium and endometriosis, tissue mRNA and protein expression of this gene were examined. We also examined the tissue expression of FABP5, another intra-cytoplasmic lipid binding protein involved in retinoid. As shown in Fig. 1, CRABP2

and FABP5 gene expression were significantly higher in endometrial tissue, with increased mRNA and protein levels noted.

Nuclear receptors mediating RA action are differentially expressed in endometrial versus endometriotic tissue and stromal cells

To determine whether the nuclear receptors RARα and RXRα were differentially expressed in endometrium and endometriosis, mRNA and nuclear protein levels of this gene were examined. As shown in Figs 1–3, RARα or RXRα mRNA levels are strikingly lower in tissues and stromal cells of endometriosis compared with those in endometrium.

Figure 2 (A) Retinol uptakes takes place predominantly in stromal cells when compared with epithelial cells. (B) There is differential mRNA expression in many genes involved in retinol uptake, metabolism and signaling in normal endometrial stromal cells (ESC) when compared with endometriotic stromal cells (OSIS) *P < 0.05; NS, non-significant.

Figure 3 The differential expression of genes involved in RA signaling is confirmed with western blotting of stromal cells. ESC, normal endometrial stromal; OSIS, endometriotic stromal cells. (A) Western blot of genes involved in RA uptake, metabolism. (B) Western blot of nuclear receptors. (C) Histogram of quantified western blots with intensities relative to actin; *P < 0.05.

Because it has been previously showed that RA can be shuttled to another nuclear protein, namely PPARβ/δ, this gene was also examined. Similar to what was found with the other nuclear receptors, PPARβ/δ mRNA and protein expression were decreased in tissue from endometriotic tissues (Fig. 1). However, in stromal cells, there was no difference noted at the mRNA level (Figs 2 and 3). The differential expression of these nuclear receptor genes, together with the altered ratios of FABP5 and CRABP2 in endometriosis, may contribute

to the resistance to apoptosis seen in endometriosis (Beliard *et al.*, 2004; Pavone *et al.*, 2010).

CYP26B1 is differentially expressed in endometrial versus endometriotic tissue and stromal cells

We next looked at the expression of CYP26A1 and CYP26B1 in normal and endometriotic tissue. Although CYP26A1 has been

Figure 4 We hypothesize that there is a differential expression of genes involved in retinol uptake and metabolism in OSISs when compared with ESCs, suggesting alterations in RA action. This could lead to the differences in both autocrine and paracrine actions of RA seen in endometriosis.

detected in the endometrium, we were only able to detect its mRNA in whole tissue samples of healthy endometrium, and protein levels were undetectable by Western blot (data not shown). In contrast, CYP26B1 was more readily detected at both the mRNA and protein level. As shown in Figs 1 and 2, this gene was differentially expressed in endometrial and endometriotic tissue and stromal cells, with higher expression in endometriosis. Higher CYP26B1 levels may lead to increased retinoid clearance in endometriotic tissues and cells, further suppressing RA levels.

Discussion

Chordate development hinges upon accurate signaling through the metabolites of vitamin A. The majority of these signals are carried out by RA, and the carefully graded control of the enzymes responsible for metabolizing RA are required for the patterning and growth

of tissues throughout an organism’s lifetime (Schug et al., 2007, 2008; Tang and Gudas, 2011). RA has been established as a potent modulator of gene transcription, through which it directs control over cell growth, differentiation and apoptosis. Our previous work indicated that RA production in endometriotic tissue is reduced relative to healthy endometrium, resulting in blunted estrogen metabolism; however, the pathways leading to this defect were unknown. Here we have characterized the expression of the genes responsible for retinoid uptake, synthesis and degradation in both normal and endometriotic tissues (Fig. 4).

Table 1 summarizes our findings, and outlines the perturbation in retinol uptake and metabolism that most likely compromises retinoid action in endometriotic stromal cells when compared with stromal cells of normal endometrium during the proliferative phase of the menstrual cycle. We chose to study tissue samples in the proliferative phase because we previously showed that PR regulated STRA6 and

Table 1 There is a differential gene expression of multiple genes involved in the retinoic acid signaling cascade in normal endometrium when compared with endometriotic tissues.

Gene name	Gene description	Fold-change	P-value
STRA6	Stimulated by retinoic acid gene 6	−0.047	<0.05
CRBP1	Cellular retinol binding protein 1	−0.076	<0.05
ALDH1A2	Aldehyde dehydrogenase 1 family, member A2	−0.524	<0.05
CRABP2	Cellular retinoic acid binding protein 2	−0.042	<0.05
FABP5	Fatty acid binding protein 5	−0.300	<0.05
CYP26B1	RA metabolizing member B1 of P450 family	+14.567	<0.05
RARα	Retinoid receptor alpha	−0.171	<0.05
RXRα	Retinoid X receptor alpha	−0.048	<0.05
PPARβ/δ	Peroxisome-proliferator-activated receptor β/δ	−0.100	<0.05

CRABP2 in a ligand-independent fashion (Pavone *et al.*, 2010). The retinoid signaling pathway first involves retinol uptake into the cell, and then its conversion to RA. We have shown that both the uptake of retinol into cells via STRA6 and the conversion to the biologically active form RA via ALDH1A2 are deficient in endometriosis. This ultimately leads to a decrease in the amount of RA available to these cells for both autocrine and paracrine actions, including epithelial induction of HSD17B2. We believe that this may contribute to both increased estradiol presence, via decreased induction of HSD17B2 and the inappropriately reduced apoptosis through altered CRABP2/FABP5 shuttling, both of which are characteristic of endometriosis (Beliard *et al.*, 2004; Bulun *et al.*, 2006; Wu *et al.*, 2008; Nasu *et al.*, 2009). As we have shown previously, the anti-apoptotic state is, in part, mediated by a decreased ratio of CRABP2 to FABP5 relative to what we observe in normal endometrial cells, where RA promotes differentiation and apoptosis via CRABP2 by shuttling RA to RAR α . Here we find that not only are the levels of CRABP2 diminished, but that there is also a reduced expression of nuclear receptors RAR α and RXR α . We previously observed that RAR α and RXR α are more integral within their respective families in endometrium and endometriosis, which is why we focused our analysis on these two receptors (Cheng *et al.*, 2006, 2007). Taken together, this suggests that the reduced apoptosis seen in endometriosis may be partially explained from both a loss in the specific expression of these nuclear receptors as well as reduced levels of their normal ligand.

In addition to differences in retinol uptake, we uncovered strikingly different levels of the genes directly responsible for RA synthesis and degradation. ALDH1A2 expression, which was decreased in endometriosis, is consistently associated with the spatio-temporal patterns of RA synthesis and signaling. Disrupting ALDH1A2 in the mouse leads to embryonic death and phenotypes of serious vitamin A deficiency. Some of these disturbances can be rescued by maternal administration of RA, suggesting that ALDH1A2 is the primary enzyme responsible for RA synthesis (Niederreither *et al.*, 1999; Deng *et al.*, 2003). In contrast, we saw increased levels of CYP26B1 accompanying endometriosis. The CYP26 family of cytochrome P450 enzymes oxidizes all-trans RA metabolites, which are less biologically active and more easily eliminated by cells (Taimi *et al.*, 2004; Ocaya *et al.*, 2010). The increased production of CYP26 enzymes is conventionally thought to protect cells from exposure to improper concentrations of RA. In endometriotic cells, we predict that increased CYP26B1 and decreased RA production strongly limit RA availability. Similar profiles for these groups of enzymes have been demonstrated in breast and ovarian cancers, as well as in leiomyomas, and in these instances it was postulated that increased expression of these enzymes may be associated with decreased apoptosis (Van heusden *et al.*, 1998; Ozpolat *et al.*, 2002; Osanai and Petkovich, 2005; Catherino and Malik, 2007). Notably, we were unable to quantify differences in CYP26A1 in our samples. CYP26A1 has been shown to be decreased in endometriotic tissues in other micro-arrays, and *in situ* studies have demonstrated its expression in the endometrial epithelium (Aghajanova *et al.*, 2011). While mRNA levels were detectable in whole tissue samples from normal endometrium, we could not detect protein expression in any samples (data not shown). Similarly, we did not observe CYP26A1 mRNA in the cultured stromal cells, suggesting that this enzyme is preferentially expressed in epithelial cells and not stromal cells.

Similar to the results of our study, Catherino and Malik (2007) reported a difference in the expression of several genes involved in

the RA signaling pathway in a leiomyoma (Malik *et al.*, 2008). Like endometriosis, leiomyomata are characterized by a decrease in apoptosis. Since tumor growth depends on a balance between cell death and cellular proliferation, Martel *et al.* (2004) speculated that decreased apoptosis may be responsible for leiomyoma pathogenesis. We believe that alterations in apoptosis may also be involved in the pathogenesis of endometriosis (Beliard *et al.*, 2004). Although the specific targets of RA are not yet known in these diseases, we speculate that the effects of RA-deficiency in their cells are likely compounded by the increased effects of estrogen. In breast cancer, RA signaling through RAR α is thought to function antagonistically to E2 signaling through estrogen receptor (ER) α , in part due to both transcription factors frequently coinciding or even competing to regulate target genes (Tang and Gudas, 2011). The specific interplay between ER and RAR function in endometriosis will be important to understanding how these cells escape apoptosis.

One limitation of our study has been the inability to directly measure retinol uptake and RA *in vivo*. Because of the limited quantities of tissue available, we are presently unable to quantify RA in our tissue samples. Studies in other biological systems have shown a close correlation between the levels of ALDH1A2 mRNA and protein and RA production (Deng *et al.*, 2003). We hypothesize that since there is a decrease in the expression of genes controlling both retinol uptake and conversion to the biologically active RA, and an increase in the expression of genes that metabolize RA to forms that can be easily excreted, decreased quantities of RA would be present in endometriotic tissues and cells.

In summary, the decreased expression of the genes involved in RA synthesis and trafficking indicates that this signaling pathway plays a central role in the pathogenesis of endometriosis (Fig. 4). A reduced amount of active RA is the result of both an underexpression of STRA6, which is predominantly expressed in the stroma and controls retinol uptake, as well as a decrease in ALDH1A2, which converts retinol to RA. Additionally, there is a decrease in the expression of CRABP2, which we have previously demonstrated, is integral in survival pathways in endometrium and endometriosis (Pavone *et al.*, 2010). There is also a higher level of CYP26B1 that converts RA to an inactive form that can be secreted by cells, further diminishing active RA levels and impacting apoptosis. The reduced expression of nuclear receptors RAR and RXR in endometriosis could also contribute to differences in biologic processes, including apoptosis and differentiation.

This work suggests that reduced expression of genes involved in the RA signaling pathway could alter the apoptosis/survival pathways in these cells. The clinical significance of retinoids in the prevention and treatment of cancer have been well established (Molin *et al.*, 1987; Bollag *et al.*, 1994; Nandan, 2006). Because retinoids regulate growth and differentiation, they have been successfully used in the treatment of various cancers. Control of this pathway could result in novel therapies that alter endometriotic cell survival and limit symptoms associated with this disease (Schug *et al.*, 2008).

Authors' roles

All authors have met the criteria for justification of authorship. They have all substantially contributed to the conception/design/acquisition of data/analysis and interpretation of data; drafted or revised the article for important intellectual content; approved the version to be published.

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