Progesterone Resistance in Endometriosis Is Modulated by the Altered Expression of MicroRNA-29c and FKBP4

Niraj R. Joshi,[1] Eduardo H. Miyadahira,[2] Yalda Afshar,[3] Jae-Wook Jeong,[1] Steven L. Young,[4] Bruce A. Lessey,[5] Paulo C. Serafini,[6] and Asgerally T. Fazleabas[1]

[1]Department of Obstetrics, Gynecology and Reproductive Biology, Michigan State University, Grand Rapids, Michigan 49503; [2]Huntigton Medicina Reprodutiva, São Paulo 04021-000, Brazil; [3]Department of Obstetrics and Gynecology, University of California, Los Angeles, California 90095; [4]Department of Obstetrics and Gynecology, The University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599; [5]Greenville Hospital System, University of South Carolina School of Medicine, Greenville, South Carolina 29605; and [6]Discipline of Gynecology, Department of Obstetrics and Gynecology, Hospital das clinicas, faculdade de Medicina, Universidade de São Paulo, São Paulo 01246-903, Brazil.

Context: Endometriosis results in aberrant gene expression in the eutopic endometrium (EuE) and subsequent progesterone resistance. MicroRNA (miR) microarray data in a baboon model of endometriosis showed an increased expression of miR-29c.

Objectives: To explore the role of miR-29c in progesterone resistance in a subset of women with endometriosis.

Design: MiR-29c expression was analyzed in the endometrium of baboons and women with or without endometriosis. The role in progesterone resistance and decidualization was analyzed by transfecting human uterine fibroblast cells with miR-29c.

Patients: Subjects diagnosed with deep infiltrative endometriosis (DIE) by transvaginal ultrasound with bowel preparation underwent surgical excision of endometriosis. Eutopic secretory endometrium was collected pre- and postoperatively. Women with normal EuE and without DIE served as controls.

Results: Quantitative reverse transcription polymerase chain reaction demonstrated that miR-29c expression increased, while the transcript levels of its target, FK506-binding protein 4 (FKBP4), decreased in the EuE of baboons following the induction of endometriosis. FKBP4 messenger RNA and decidual markers were statistically significantly decreased in decidualized human uterine fibroblast cells transfected with a miR-29c mimic compared with controls. Human data corroborated our baboon data and demonstrated higher expression of miR-29c in endometriosis EuE compared with normal EuE. MiR-29c was significantly decreased in endometriosis EuE postoperatively compared with preoperative tissues, and FKBP4 showed an inverse trend following radical laparoscopic resection surgery.

Conclusions: We demonstrate that miR-29c expression is increased in EuE of baboons and women with endometriosis, which might contribute to a compromised progesterone response by diminishing the levels of FKBP4. Resection of DIE is likely to reverse the progesterone resistance associated with endometriosis in women. **(*J Clin Endocrinol Metab* 102: 141–149, 2017)**

Endometriosis affects ~10% women of reproductive age with a prevalence of 20% to 50% in infertile women and >70% in women with pelvic pain (1, 2). This gynecological disorder is characterized by the presence of ectopic endometrial tissue containing glands and stroma (3, 4). The etiology and natural history of endometriosis

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Abbreviations: DIE, deep infiltrative endometriosis; EPC, estrogen + medroxyprogesterone acetate + dibutyryl-cAMP treatment; EuE, eutopic endometrium; FKBP4, FK506-binding protein 4; HOXA10, homeobox A10; HuF, human uterine fibroblast; MIG6, mitogen-inducible gene 6; miR, microRNA; miR-29c, microRNA-29c; mRNA, messenger RNA.

remain unclear. Sampson's theory of retrograde menstruation, the most widely accepted etiology, proposes that menstrual tissue passing through the fallopian tube attaches to extrauterine peritoneum and grows under the influence of ovarian steroids (5). The actions of estrogen and progesterone are mediated through their cognate steroid receptor, which are expressed in both eutopic and ectopic endometrium (6). Gene array studies from our laboratory have demonstrated that ectopic endometriotic lesions alter eutopic endometrial gene expression as early as 1 month after disease induction in baboons, and these changes are similar to those seen in women with endometriosis (4, 7, 8). These data suggest that the eutopic endometrium (EuE) is initially characterized by a dominance of estrogen action (9, 10) and then followed by the development of progesterone resistance, reflected by decreased expression of progesterone-induced genes (7, 11, 12). The molecular mechanism responsible for the progesterone resistance remains unclear.

MicroRNA (miR) are single-stranded noncoding RNA molecules approximately 22 nucleotides in length that function as repressors of gene expression through messenger RNA (mRNA) cleavage and translational repression (13). Studies demonstrated that miR plays a role in the human reproductive physiology, and abnormal expression of miR has been observed in several diseases of the female reproductive tract, including endometriosis (14–19). Our study on miR-451 demonstrated a role of this miR in modulating cell proliferation (16). Hawkins *et al.* (20) have demonstrated a functional role for miR-29c dysregulating extracellular matrix genes in endometriomas. Previous work from our baboon studies demonstrated that a number of miRs are altered in the baboon model of endometriosis, including one of the most highly upregulated miRs in response to disease induction, miR-29c (16). *In silico* analysis demonstrated a miR-29c consensus binding site in the 3′-UTR of the FK506-binding protein 4 (FKBP4) gene, which affects embryonic implantation and decidualization (21, 22) and expression of which has been shown to be altered in the EuE of women and baboons with endometriosis (11, 23). Overexpression of miR-29c in human uterine fibroblast (HuF) cells inhibits decidualization (20), a process attenuated in endometriosis (12, 24, 25).

This study was designed to investigate the hypothesis that elevated miR-29c levels suppress FKBP4 expression in the EuE of baboons and women with endometriosis, contributing to progesterone resistance. To support our *in vivo* findings, a series of *in vitro* experiments were performed. HuF cells were transfected with a miR-29c mimic and subjected to an *in vitro* decidualization stimulus. FKBP4 levels, markers of progesterone action [decorin, homeobox A10 (HOXA10), and mitogen-inducible gene 6 (MIG6)], and decidualization (prolactin) were assessed. Because excision of deep infiltrative endometriosis (DIE) before *in vitro* fertilization improves pregnancy rates (26), we also compared miR-29c and FKBP4 expression in women with DIE pre- and postoperatively.

Materials and Methods

Induction of experimental endometriosis and collection of baboon tissues

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois, Chicago and Michigan State University. Endometriosis was experimentally induced in 3 female baboons (*Papio anubis*) by intraperitoneal inoculation with menstrual tissue on 2 consecutive menstrual cycles, as previously described (8, 27). In the cycle prior to the induction of endometriosis, control EuE (n = 3) was obtained at laparotomy on day 10 postovulation. Endometriosis was then induced in the same 3 animals by intraperitoneal inoculation of autologous menstrual tissue on 2 consecutive cycles. Following laparoscopic confirmation of endometriosis, EuE samples (n = 3) were obtained at 3 months postinduction of disease. At 15 to 16 months following the second inoculation, the animals (n = 3) were euthanized as required by the Institutional Animal Care and Use Committee, which permits a maximum of 4 invasive surgeries, and a necropsy was carried out to obtain all of the associated reproductive tissues within the peritoneal cavity. Samples were snap-frozen in liquid nitrogen for molecular analysis.

Patient samples

We obtained institutional review board approval from the School of Medicine of the University of São Paulo. Informed consent was obtained from patients. Patients were divided according to the result of the transvaginal ultrasound with bowel preparation (n = 26). Fifteen women with DIE underwent laparoscopic resection of the ectopic tissue, and 11 patients without DIE served as the control group. No statistical difference between the groups was observed in age, body mass index, basal follicle-stimulating hormone, basal estradiol, previous *in vitro* fertilization failure, and repeated abortion. Endometrial samples were collected from both groups. In the endometriosis group, samples were obtained before (Eosis preoperative) and after (Eosis postoperative) the surgery. Furthermore, ectopic tissue (Lesions) collected during the surgical intervention was also included in the analysis. Endometrial samples were obtained with a Pipelle curette (Pipelle de Cornier; Laboratoire C.C.D., Paris, France) and stored in RNAlater at −80°C. Only midsecretory phase samples were included. The groups were Eosis preoperative (n = 9), Eosis postoperative (n = 4), and control (n = 8).

Deep infiltrative endometriosis surgery

After preoperative evaluation, endometriotic lesions were laparoscopically excised. Surgeries were all performed by 2 experienced gynecological surgeons (14 and 10 years of experience) using similar techniques (*i.e.*, scissors and diathermy) to remove all superficial and deep endometriotic lesions. These

tissues were then examined histologically for the presence of disease.

Immunohistochemistry

Immunohistochemical staining for FKBP4 protein was performed using formalin-fixed, paraffin-embedded endometrial samples as previously described (11). Staining was analyzed at 20× using a Nikon (Tokyo, Japan) microscope. Assessment of staining intensity and distribution was via a semiquantitative H-score system by a single blinded observer (11, 28).

Isolation and culture of primary stromal cells

Primary eutopic endometrial stromal cells were isolated from the endometrial biopsy specimens from normally cycling women (n = 4) and women with endometriosis (n = 4) at both the University of North Carolina (Chapel Hill, NC) and the Greenville Hospital System (Greenville, SC) with informed consent (Supplemental Table 2). After trypsinization, dissociated cells were maintained and propagated in defined medium (phenol red–free RPMI 1640) with or without 10% fetal bovine serum. These cells isolated from each individual patient represent a proliferating population of nondifferentiated stromal cells and were used between passages 3 and 5 (12).

Isolation and culture of primary HuFs

HuF cells were isolated from the decidua parietalis, dissected from the placental membranes after vaginal delivery from 3 different individual subjects. Stromal cells were isolated as previously described (29). The institutional review board of Michigan State University approved these studies.

In vitro decidualization

Decidualization was induced in HuF cells (in triplicate) at 80% to 90% confluence in phenol red–free RPMI-1640 medium supplemented with 2% charcoal dextran stripped fetal bovine serum by treatment with EPC (0.5 mM dibutyryl-cAMP, 36 nM 17β-estradiol, and 1 μM medroxyprogesterone acetate in ethanol) for up to 8 days (12). Controls were treated with vehicle and the media were changed every 2 days.

RNA isolation and quantitative reverse transcription polymerase chain reaction

Total RNA was isolated using Trizol reagent (Invitrogen, Waltham, MA), and RNA quality check was performed using NanoDrop 2000 (Thermo Scientific, Waltham, MA). For measuring miR transcript levels in *in vivo* and *in vitro* samples, 100 ng total RNA was reverse transcribed using the TaqMan miR reverse transcription kit (Applied Biosystems, Foster City, CA) as per the manufacturer's protocol and as previously described (16). For mRNA analysis, total RNA (1000 ng) was reverse transcribed using the High-Capacity cDNA Synthesis Kit (Applied Biosystems). After complementary DNA synthesis, the quantitative reverse transcription polymerase chain reaction was carried out for genes using specific primers (Supplemental Table 3) for FKBP4, decorin, HOXA10, MIG6, prolactin, and 18S (endogenous control) with SyBrGn PCR Master Mix (Applied Biosystems). All quantitative reverse transcription polymerase chain reactions were run for 40 cycles, and fold change was calculated using $^{\Delta\Delta}$Ct method (30).

3′ -UTR luciferase assay

HuF cells were seeded in triplicate and allowed to attach for 16 hours. The cells were then cotransfected with 2 plasmids and a miR mimic using Lipofectamine RNAiMax reagent (Invitrogen). 3′-UTR luciferase assay was performed using the Dual Luciferase Reporter Assay Kit (Promega, Madison, WI) and relative luciferase activity was measured according to the manufacturer's protocol and as described previously (16). Three independent experiments were performed and data are presented as the mean ± SD.

Statistical analysis

Differences in miR and gene expression were compared following normalization against U6 and 18S, respectively. One-way analysis of variance was used to test the null hypothesis of group difference followed by Sidak's multiple comparisons test. For *in vitro* studies, miR and gene expression was normalized against U6 and 18S. For data comparing only 2 groups, a nonparametric Mann-Whitney *U* test was used. GraphPad Prism 7.0 statistical software (GraphPad Software, La Jolla, CA) was used for analyzing the data.

Results

Induction of endometriosis leads to increased expression of miR-29c and decreased expression of its predicted target, FKBP4, in a baboon model of endometriosis

In the current study, we evaluated miR-29c expression by quantitative reverse transcription polymerase chain reaction and observed that the eutopic baboon endometrial miR-29c is upregulated ~3-fold and ~7-fold ($P < 0.001$) at 3 and 15 months after endometriosis induction, respectively [Fig. 1(A)]. To predict potential targets of miR-29c, TargetScan 6.2 software (Whitehead Institute for Biomedical Research, Boston, MA) (31, 32) resulted in 1238 potentially conserved targets, which included FKBP4. We focused on FKBP4, as we and others have demonstrated that FKBP4 plays a key role in endometrial progesterone action, and FKBP4 expression is decreased in EuE of baboons with endometriosis (11, 21, 33). FKBP4 mRNA was suppressed at 3 and 15 months in the eutopic endometrium of baboons with endometriosis compared with controls [Fig. 1(B)]. Decreased FKBP4 expression correlated with decreased expression of the progesterone-induced gene decorin ($P < 0.05$) in these samples [Fig. 1(C)] (34).

FKBP4 expression is decreased in women with endometriosis

Immunohistochemistry [Fig. 2(A)] with H-score [Fig. 2(B)] analysis for FKBP4 protein in women with and without endometriosis was consistent with our previously reported baboon data (11) and in EuE of women with endometriosis (23). Although signal intensity for FKBP4 protein in both glandular epithelial cells and stromal cells decreased, this was more prominent in EuE stromal cells.

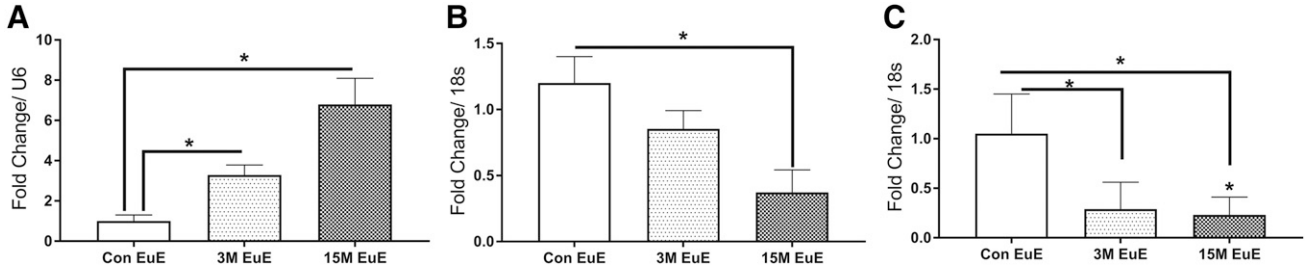

Figure 1. Quantitative reverse transcription polymerase chain reaction analysis of miR-29c, FKBP4, and decorin expression in baboon tissues. (A) miR-29c expression was significantly increased in the eutopic endometrial biopsy specimens obtained during the midsecretory phase from baboons (n = 4) with endometriosis compared with controls (n = 3). Decreased expression of (B) FKBP4 and (C) decorin in the EuE of baboons following the induction of endometriosis. Data displayed as mean ± SD and analyzed by 1-way analysis of variance followed by Sidak's multiple comparisons test (*$P < 0.05$). Con, without endometriosis; M, months.

MiR-29c regulates the expression of FKBP4 and progesterone-regulated genes *in vitro*

To investigate the mechanism underlying the inverse relationship of miR-29c and FKBP, we cotransfected HuF cells with a firefly luciferase expression vector containing the 3′-UTR of FKBP4, Renilla luciferase expression plasmid (internal control), and miR-29c mimic or nontargeting negative controls. Results show a significant decrease in luciferase activity in the HuF cells cotransfected with miR-29c mimic compared with the nontargeting negative controls [Fig. 3(A)], suggesting that miR-29c directly targets 3′-UTR of FKBP4. To evaluate the effect of miR-29c overexpression on FKBP4 expression and progesterone-regulated genes, we transfected HuF cells with miR-29c mimics or nontargeting negative controls and treated the cells with vehicle or progesterone (1μM medroxyprogesterone acetate) for 48 hours. Cells transfected with the miR-29c mimics demonstrated an increase in miR-29c abundance [Fig. 3(B)] compared with the cells transfected with nontargeting negative controls, confirming transfection efficiency. Progesterone treatment resulted in increased levels of FKBP4 [Fig. 3(C)], decorin [Fig. 3(D)], HOXA10 [Fig. 3(E)], and MIG6 [Fig. 3(F)] in nontargeting negative control-transfected HuF cells. In contrast, we observed a significant reduction in the progesterone-regulated genes (decorin, HOXA10, and MIG6) in HuF cells transfected with miR-29c mimic compared with the nontargeting negative control-transfected cells treated with progesterone. The known role of FKBP4 in progesterone action and its suppression by miR-29c suggests that effects of miR-29c on other progesterone target genes (decorin, HOXA10, and MIG6) are mediated by decreased FKBP4 expression. These *in vitro* studies support the inverse correlation observed *in vivo* between miR-29c and FKBP4 expression and its effect on progesterone-regulated gene expression.

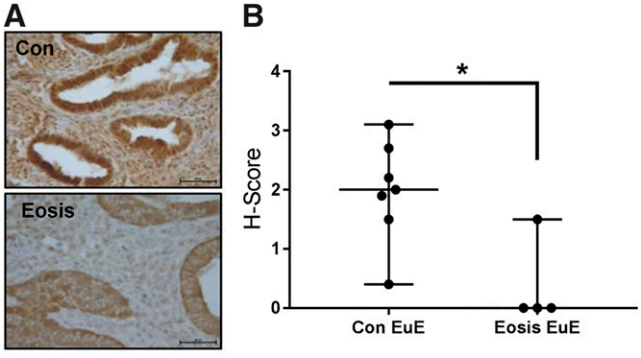

Figure 2. Immunohistochemical and H-score analysis of FKBP4 protein expression in the eutopic endometrium of women. (A) Immunohistochemistry results show a decreased signal intensity for FKBP4 protein in both endometrial glandular epithelium and stroma of women with endometriosis compared with the women without disease. (B) Subsequent H-score analysis shows a significant decrease in FKBP4 protein expression in women with endometriosis (n = 7) compared with controls (n = 4). Data displayed as mean ± SD and analyzed by performing the Mann-Whitney test (P = 0.0152). Bar = 50 μm. Con, without endometriosis; Eosis, endometriosis.

Overexpression of miR-29c alters the *in vitro* decidualization response

Our *in vivo* and *in vitro* data demonstrate that increased miR-29c results in decreased expression of FKBP4. To explore the possible biological effect of increased expression of miR-29c, we performed *in vitro* decidualization experiments using HuF cells, transfected with either miR-29c mimic or nontargeting negative controls exposed to a decidualization stimulus (EPC). Transfection with the miR-29c mimic resulted in an increased detection of miR-29c [Fig. 4(A)] and this was unaffected by EPC treatment. Treatment of nontargeting control-transfected cells with EPC also induced decorin, HOXA10, and prolactin but had little effect on FKBP4 [Fig. 4(B–F)]. Cells transfected with miR-29c mimic and EPC had reduced FKBP4 expression associated with reduced decorin, HOXA10, and MIG6 [Fig. 4(B–E)] and reduced expression of the decidualization marker, prolactin, compared with cells transfected with a nontargeting control. These data demonstrate that increased expression of miR-29c

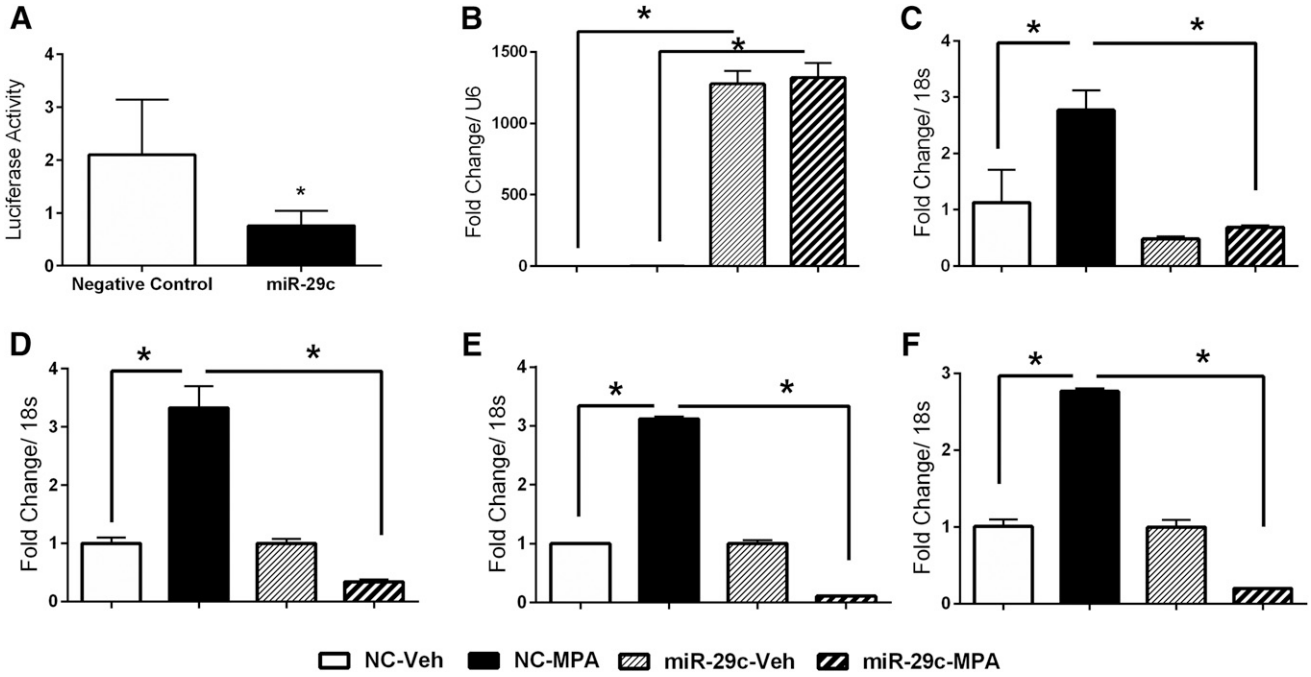

Figure 3. *In vitro* results shows the inverse relationship between miR-29c and FKBP4. (A) The Luciferase data are normalized with the Renilla and show that FKBP4 is a direct target of miR-29c. The Luciferase construct containing wild-type 3′-UTR of FKBP4 was cotransfected with either miR-29c mimic or nontargeting negative control in human uterine fibroblast cells along with Renilla plasmid (internal control). Quantitative reverse transcription polymerase chain reaction data suggest that overexpression of (B) miR-29c decreases (C) FKBP4 levels and attenuates the progesterone signaling as evident by the compromised expression of (D) decorin, (E) homeobox A10, and (F) mitogen-inducible gene 6. Mean values with SD are shown (*$P < 0.05$). miR-29c, microRNA-29c mimic-transfected cells; MPA, medroxyprogesterone acetate treatment; NC, nontargeting negative control-transfected cells; Veh, vehicle treatment.

compromises the decidualization response and is likely mediated by decreased levels of FKBP4.

EuE stromal cells from women with endometriosis have a dampened decidualization response

Primary stromal cells from women with and without endometriosis were exposed to the EPC. We demonstrated an increase in HOXA10 [Fig. 5(B)] and prolactin [Fig. 5(C)] expression in stromal cells collected from women without endometriosis. In contrast, we observed a significant decrease in FKBP4 [Fig. 5(A)] and HOXA10 [Fig. 5(B)] transcript levels in decidualized stromal cells from women with endometriosis compared with the stromal cells from women without endometriosis, similar to the findings in HuF cells. We also observed a decrease in prolactin [Fig. 5(C)] levels, suggesting a blunted decidualization response in endometriosis; however, this decrease was not statistically significant due to variability in the decidualization response among the stromal cells.

The effect of lesion excision on miR-29c and FKBP4 expression in EuE of women with endometriosis

There was a significant upregulation of miR-29c and a decrease in FKBP4 mRNA expression in EuE biopsy specimens obtained in the midsecretory phase from women with endometriosis compared with controls (Fig. 6). Laparoscopic excision of DIE significantly decreased the expression of miR-29c and restored the expression of FKBP4 transcript levels in the EuE 6 months postsurgery (Fig. 6).

Discussion

Endometriosis is characterized by severe cyclic chronic pelvic pain and infertility. Although the causes of endometriosis-associated infertility are not well defined, the presence of the disease results in aberrant gene expression in the EuE and the development of progesterone resistance (1, 2, 7). Rodent models suggest that FKBP4 plays a critical role in progesterone action on the endometrium necessary for embryonic implantation (35, 36). It is well established that progesterone signaling drives the decidualization process, which is crucial for implantation in both rodents and primates (37). Both human and animal studies have confirmed that aberrant progesterone action, despite normal progesterone levels, compromises fertility in the presence of endometriosis (11, 23). These observations emphasize the importance of FKBP4 as a physiological cochaperone for progesterone signaling in the uterus and suggests that progesterone resistance, a hallmark of endometriosis, can be attributed, in part, to decreased FKBP4 expression (11, 21, 23,

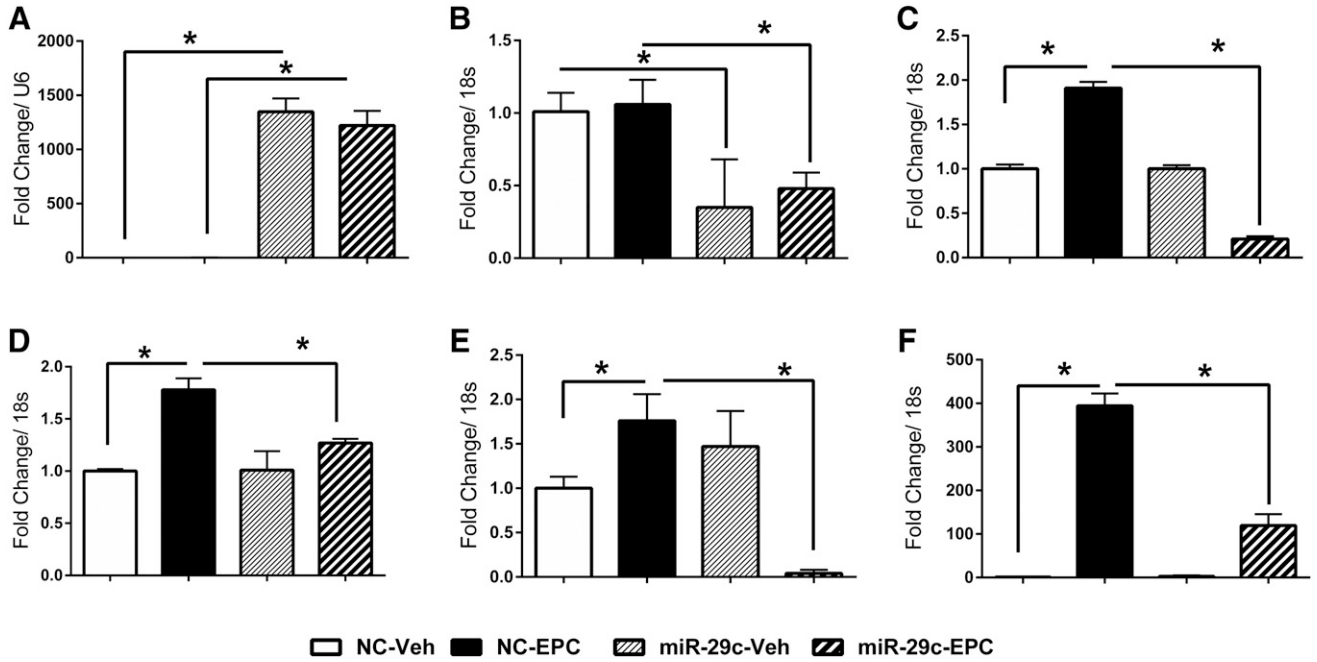

Figure 4. Quantitative reverse transcription polymerase chain reaction data suggest that overexpression of miR-29c altered the decidualization response. Effect of miR-29c mimics transfection on (A) miR-29c, (B) FKBP4, (C) decorin, (D) homeobox A10, (E) mitogen-inducible gene 6, and (F) prolactin transcript levels in human uterine fibroblast cells exposed to decidualization mixture for 6 days. Mean values of 3 separate experiments with SD are shown (*$P < 0.05$). miR-29c, microRNA-29c mimic-transfected cells; MPA, medroxyprogesterone acetate treatment; NC, nontargeting negative control-transfected cells; Veh, vehicle treatment.

33, 38). We recently reported microarray data demonstrating that endometriosis induction in the baboon results in altered expression of a number of miRs, including miR-29c (16). In the current study, we focused on the expression of miR-29c and one of its predicted targets, FKBP4.

Our data suggest that HuF cells transfected with a miR-29c mimic demonstrated a significantly attenuated decidualization response. These results complement previously reported data that the overexpression of miR-29c leads to a blunted decidualization response associated with a decrease in the expression of Insulin-like Growth Factor Binding Protein-1 and WNT4 (20). This validates the observation in rodents and demonstrates that the decrease in FKBP4 in baboons and women with endometriosis is associated with impaired decidualization and embryo-maternal interactions and implantation (35).

Decorin is a member of the proteoglycan family reported to modulate the effects of the receptor tyrosine kinase family and hepatocyte growth factor receptors (39–41). Decorin expression is upregulated in stromal cells following the induction of decidualization (42), and recent reports suggest that progesterone induces the expression and secretion of decorin in endometrial cells (34). *In vivo* studies have also suggested that treatment with a strong progestin, dienogest, promoted the increase in decorin expression in ovarian endometriosis lesions (34). We observed increased decorin expression in progesterone-treated and decidualized HuF cells transfected with nontargeting negative control. In HuF cells transfected with miR-29c and treated with progesterone or the decidualization mixture, we observed a significant decrease in decorin expression compared with the nontargeting negative controls. These observations further support our hypothesis that increased expression of miR-29c results in a progesterone-resistant environment both *in vivo* and *in vitro*.

MIG6 expression is induced by progesterone and plays an important role in inhibiting estrogen signaling in the uterus (22, 43, 44). Our *in vitro* observation suggests a significant decrease in MIG6 expression in HuF cells overexpressing miR-29c and treated with either progesterone or the decidualization mixture. This observation also leads us to suggest that the increased expression of miR-29c might also be responsible for estrogen dominance observed in endometriosis pathology since MIG6 is an important inhibitor of estrogen signaling (22).

HOXA10 expression is upregulated during the window of implantation in fertile women (45), but this increase in HOXA10 is inhibited in the EuE with endometriosis (12, 46, 47). We speculate that the compromised expression of HOXA10 might be due to the increased expression of miR-29c and subsequent progesterone resistance. It has been reported that the

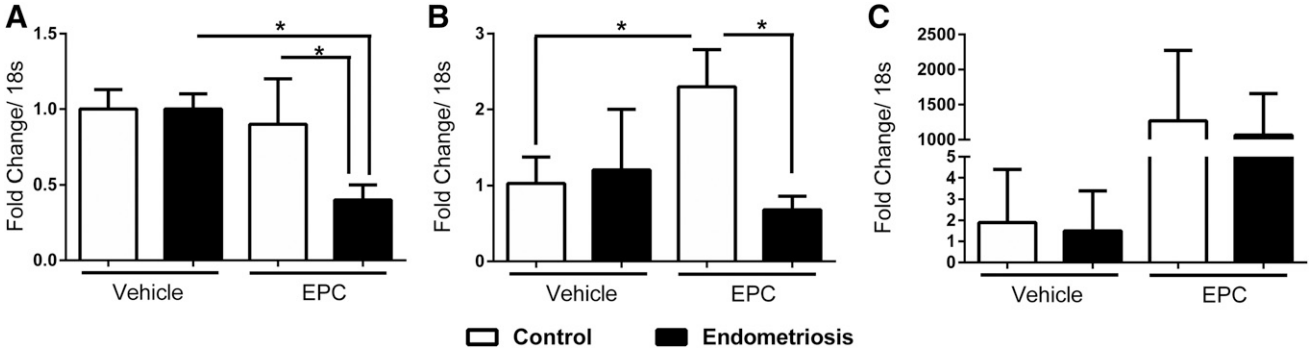

Figure 5. Quantitative reverse transcription polymerase chain reaction of the decidualization response in stromal cells from women with endometriosis compared with women without endometriosis. The data show that stromal cells from women with endometriosis have lower levels of (A) FKBP4, (B) homeobox A10, and (C) prolactin when exposed to decidualization mixture compared with stromal cells from women without endometriosis. Mean values with SD are shown ($*P < 0.05$).

expression of endometrial HOXA10 is restored postoperatively in gynecological pathologies such as endometriomas, leiomyomas, and hydrosalpinx (48, 49), which correlates with our postoperative finding on miR-29c, FKBP4, and HOXA10 expression in the EuE of women with DIE.

It is well established that endometriosis is one of the major causes of infertility and inflammation in women and that surgical resection of endometriotic lesions not only provides symptomatic relief but also improves fecundity (26, 50). The molecular changes in the eutopic endometrium underlying postoperative intervention remain elusive. To our knowledge, few studies suggest that surgical removal of lesions alters local and systemic proinflammatory cytokines (50, 51) and periimplantation endometrial HOXA10 expression (48) in patients with endometriosis. In this study, we demonstrate that the excision of endometriotic lesions restores the FKBP4 expression and a progesterone-responsive environment in the eutopic endometrium via a decreased expression of miR-29c expression. These observations are also supported by the fact that 3 of 4 women in the postoperative group analyzed in this study who underwent subsequent *in vitro* fertilization were able to get pregnant.

There are few limitations to the current study. The sample size for the overall study is low, and particularly for the postoperative eutopic endometrial biopsy specimens, we were only able to collect 4 samples, since the other 5 patients got pregnant following surgery and assisted reproductive therapy. Therefore, we were unable to correlate mRNA changes with protein expression by immunohistochemistry or Western blot. In the future, we plan to address these limitations and focus on studies on understanding how altered miR expression contributes toward progesterone-resistant EuE of women and baboons with endometriosis.

Our results suggest that endometriosis results in a significant increase in miR-29c and decrease in FKBP4 expression in the eutopic endometrium. The association with endometriosis was further confirmed when miR-29c expression was decreased and FKBP4 expression was restored in eutopic tissues following laparoscopic lesion ablation. Combining *in vivo* and *in vitro* data, it can be inferred that the compromised expression of FKBP4 due to the increased expression of miR-29c results in blunted progesterone signaling and might contribute to the observed progesterone resistance in women with endometriosis. Furthermore, our data suggest that the infertility in the subset of women with endometriosis who fail to conceive might be due, at least in part, to a decidualization defect as a result of decreased progesterone signaling in the absence of FKBP4.

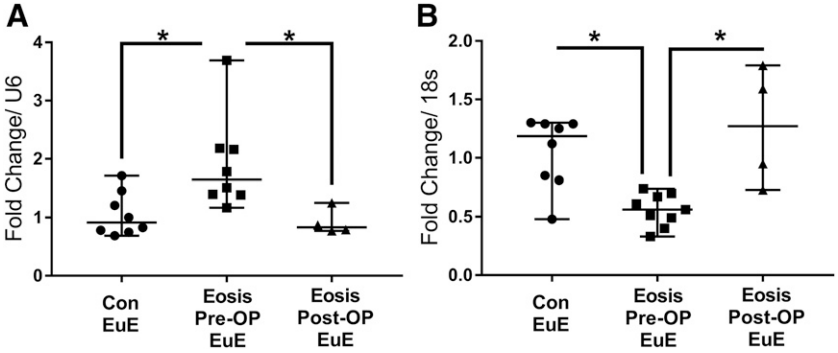

Figure 6. Quantitative reverse transcription polymerase chain reaction analysis of microRNA-29c (miR-29c) expression in human EuE. miR-29c and FKBP4 expression in midsecretory phase eutopic endometrial biopsy specimens were obtained from control and women with endometriosis before and after surgery. Data are displayed as mean ± SD and analyzed by performing the Kruskal-Wallis test followed by Dunn's multiple comparisons test ($*P < 0.05$). Con, without endometriosis; Eosis, endometriosis; Post-OP, after surgery; Pre-OP, before surgery.

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Address all correspondence and requests for reprints to: Asgerally T. Fazleabas, PhD, Department of Obstetrics and Gynecology, and Reproductive Biology, College of Human Medicine, Michigan State University, Grand Rapids, MI 49503. E-mail: asgi@hc.msu.edu

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