Endometriosis Located Proximal to or Remote From the Uterus Differentially Affects Uterine Gene Expression

Reproductive Sciences
2016, Vol. 23(2) 186-191
© The Author(s) 2015
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1933719115613449
rs.sagepub.com

Hanyia Naqvi, MS¹, Ramanaiah Mamillapalli, PhD¹,
Graciela Krikun, PhD¹, and Hugh S. Taylor, MD¹

Abstract

The mechanisms that lead to the altered uterine gene expression in women with endometriosis are poorly understood. Are these changes in gene expression mediated by proximity to endometriotic lesions or is endometriosis a systemic disease where the effect is independent of proximity to the uterus? To answer this question, we created endometriosis in a murine model either in the peritoneal cavity (proximal) or at a subcutaneous remote site (distal). The expression of several genes that are involved in endometrial receptivity (*homeobox A10* [*Hoxa10*], *homeobox A11* [*Hoxa11*], insulin-like growth factor binding protein 1 [*Igfbp1*], Kruppel-like factor 9 [*Klf9*], and progesterone receptor [*Pgr*]) was measured in the eutopic endometrium of mice transplanted with either proximal or distal endometriosis lesions. Decreased expression of *Hoxa10*, *Igfbp1*, *Klf9*, and total *Pgr* genes was observed in the eutopic endometrium of mice with peritoneal endometriosis. In the mice with distal lesions, overall expression of these genes was not as severely affected, however, *Igfbp1* expression was similarly decreased and the effect on *Pgr* was more pronounced. Endometriosis does have a systemic effect that varies with distance to the end organ. However, even remote disease selectively and profoundly alters the expression of genes such as *Pgr*. This is the first controlled experiment demonstrating that endometriosis is not simply a local peritoneal disease. Selective alteration of genes critical for endometrial receptivity and endometriosis propagation may be systemic. Similarly, systemic effects of endometriosis on other organs may also be responsible for the widespread manifestations of the disease.

Keywords

endometriosis, endometrial receptivity, uterus, progesterone resistance

Introduction

Endometriosis is an estrogen-dependent disorder that affects 5% to 10% of reproductive-age women causing pelvic pain, dysmenorrhea, and infertility.^{1,2} The defining characteristic feature of endometriosis is the ectopic growth of endometrial stromal and glandular cells outside the uterine cavity. Endometrial lesions commonly occur in the peritoneal cavity, however, they occur rarely at sites remote from the peritoneal cavity and the uterus.¹⁻³ Common explanations for the etiology and pathophysiology of endometriosis include Sampson theory of retrograde menstruation accounting for the peritoneal lesions, however, metastatic hematogenous or lymphatic spread of menstrual tissue may lead to endometriosis at distant sites.⁴⁻⁷ Further, migration, engraftment, and differentiation of bone marrow-derived stem cells have been demonstrated to lead to lesions at distant sites as well.⁸⁻¹²

Women with endometriosis often report symptoms outside the reproductive tract and separate from those associated with sites of known endometriosis. For example, endometriosis has been associated with systemic inflammation,¹³⁻¹⁷ alterations in

circulating immune cells,¹⁸⁻¹⁹ and inflammatory cytokines.²⁰⁻²² Endometriosis has been postulated to be a focal disease with systemic effects. We sought to determine in a controlled experiment whether endometriosis has systemic effects. Endometrial receptivity is altered in endometriosis.²³⁻²⁵ We and others have described alterations in the endometrial expression of genes necessary for optimal receptivity seen in women with endometriosis.²⁶⁻³² Here, we sought to determine whether endometriosis remote from the peritoneal cavity would have a systemic effect. We looked at the ability of endometriosis outside the peritoneal cavity to alter uterine gene expression

¹ Department of Obstetrics, Gynecology and Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA

Corresponding Author:

Ramanaiah Mamillapalli, Department of Obstetrics, Gynecology and Reproductive Sciences, Yale School of Medicine, 333 Cedar Street, PO Box: 208063, New Haven, CT 06510, USA.
Email: ramana.mamillapalli@yale.edu

and whether the effect would vary from that of peritoneal disease.

The *Hox* genes encode transcription factors that are involved in embryonic development and are also involved in endometriosis.³³⁻³⁷ *Hoxa10* and *Hoxa11* are essential for endometrial development during the menstrual or estrus cycle and to establish conditions necessary for embryo implantation in both humans and mice.^{32,38-42} The progesterone receptor (*Pgr*) is also clearly required for endometrial receptivity allowing embryo implantation.^{24,43} Kruppel-like factor 9 (*Klf9*), a transcriptional regulator of endometrial cell proliferation and differentiation, has been demonstrated to mediate progesterone signaling pathways in endometrial epithelial cells by its selective interaction with both *Pgr* isoforms (*Pgr A* and *Pgr B*).⁴⁴⁻⁴⁶ Similarly, insulin-like growth factor binding protein 1 (*Igfbp1*) is expressed in the late secretory phase of endometrium under the influence of the *Pgr*.⁴⁷⁻⁵¹ Insulin-like growth factor binding protein 1 regulates the pathophysiological function of *Igfbp1* in proliferation and apoptosis of endometrial cells. Aberrant expression of *Hoxa10* in endometriosis results in negative regulation of *Igfbp1* in the endometrium.⁵¹ Here, we report the expression of these genes in the eutopic endometrium of mice with endometriosis near to or remote from the endometrium. We identify both a local and a systemic effect of endometriosis on the endometrium.

Materials and Methods

Induction of Endometriosis in Mice

Six-week-old CD1 female mice were purchased from Charles River Laboratories (Wilmington, Massachusetts) and kept under regulated conditions of light and darkness for 12 hours. Donor mice were used as a source of endometrial tissue for the creation of experimental endometriosis as described previously.¹⁶ Recipient mice ($N = 8$) were divided into 2 groups, one in which we created proximal endometriosis (in proximity of the uterus, ie, in the peritoneal cavity) and a second where we created distal-site endometriosis (remote from the peritoneal cavity). Peritoneal endometriosis was created by splitting the uterine horn longitudinally to expose the lumen. The 2 sections were sutured (Polysorb 4-0; Syneture, Norwalk, Connecticut, USA) to the parietal peritoneum on contralateral sides of the recipient mouse. Control mice ($n = 8$) received identical laparotomy incisions at the ventral midline. The distal-site mouse model ($n = 8$) was created by a subcutaneous subdermal incision at the dorsal portion of the chest. The 2 uterine sections were transplanted, and incision was sutured. Control mice ($n = 6$) were created with identical subdermal incisions. After 12 weeks of endometrial growth, the whole uteri were collected from each group. The uterine horns were snap-frozen in Trizol reagent (Invitrogen, Carlsbad, California). Ethical guidelines were followed for the use of animals as established by Institutional Animal Care and Use Committee, Yale University, and the US Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research and Training.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated from the eutopic endometrium of mice using Trizol reagent (Invitrogen) and then purified with the RNeasy MinElute Cleanup Kit (Qiagen, Valencia, California) according to the manufacturer's instructions. Purified RNA (50 ng) was reverse transcribed using iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, California). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using SYBR Green (Bio-Rad Laboratories) and optimized in the MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad Laboratories). The specificity of the amplified transcript was confirmed by a melting curve analysis. The primer sequences used were described previously by Lee et al.²⁶ Expression levels of *Hoxa10*, *Hoxa11*, *Igfbp1*, total *Pgr*, and *Klf9* messenger RNA (mRNA) were quantified, and the relative expression levels were normalized to β -actin. The relative gene expression ratio was calculated using comparative C_T method ($2^{-\Delta\Delta C_T}$).

Statistics

Results are presented as mean $\pm$ standard deviation (SD). Statistical significance was determined using 1-way analysis of variance with the Newman-Keuls multiple analysis. All statistical analyses were carried out using Graph Pad Prism 4.00 for Macintosh (GraphPad Software for Science Inc, San Diego, California).

Results

Altered Gene Expression in the Eutopic Endometrium Near Proximal Endometriosis Lesions

Expression of genes mediating endometrial receptivity was measured in the eutopic endometrium by qRT-PCR after 12 weeks of endometriosis induction in the peritoneum as a model of proximal endometriosis (Figure 1). *Hoxa10* mRNA expression was decreased by 3.78-fold ($P = .04$) in the proximal model compared to the control mice without endometriosis. *Hoxa11* mRNA expression was decreased by 2.11-fold ($P = .23$). The expression of *Igfbp1* was decreased by 3.63-fold ($P = .04$) relative to the corresponding control. Kruppel-like factor 9 gene expression was decreased by 3.06-fold ($P = .03$) in the proximal endometriosis model compared to that of the control group. There was a trend toward decreased *Pgr*, however, no significant change in the mRNA levels of *Pgr* between the control and the experimental groups was observed.

Altered Gene Expression in the Eutopic Endometrium Remote From the Distal Endometriosis Lesions

To identify systemic effects of endometriosis, we measured endometrial gene expression in the distal endometriosis model. As shown in Figure 2, there was no significant change in the mRNA levels of *Hoxa10* (0.81-fold; $P = .37$) in comparison to the corresponding control group. There was a trend toward

Figure 1. Quantitative real-time polymerase chain reaction (qRT-PCR) demonstrates *Hoxa10*, *Hoxa11*, insulin-like growth factor binding protein-1 (*Igfbp1*), Kruppel-like factor 9 (*Klf9*), and total progesterone receptor (*Pgr*) gene expression in the uterus of mice with proximal endometrial lesions compared to the control mice. We identified decreased expression of *Hoxa10* (3.78-fold), *Igfbp1* (3.63-fold), and *Klf9* (3.06-fold) in the endometrium. Each bar represents mean $\pm$ standard deviation (SD) for data from 3 individual experiments, and each experiment was performed in triplicate. *Statistical significance ($P < .05$) compared to the respective control.

Figure 2. Quantitative real-time polymerase chain reaction (qRT-PCR) demonstrates *Hoxa10*, *Hoxa11*, insulin-like growth factor binding protein 1 (*Igfbp1*), Kruppel-like factor 9 (*Klf9*), and total progesterone receptor (*Pgr*) gene expression in the uterus of mice with distal endometrial lesions compared to the control mice. Expression of *Igfbp1* (1.93-fold) and total *Pgr* (12.44-fold) was decreased. Each bar represents mean $\pm$ standard deviation (SD) for data from 3 individual experiments, and each experiment was performed in triplicate. *Statistical significance ($P < .05$) compared to the respective control.

decreased *Hoxa11* mRNA expression levels (1.76-fold; $P = .08$) when compared to controls, less pronounced than demonstrated previously when the lesions are located in the peritoneal cavity. The mRNA expression levels of *Igfbp1* gene were decreased by 1.93-fold ($P = .02$) when compared to controls. In contrast to *Igfbp1* gene expression, the *Klf9* gene expression was not significant relative to the control group (reduced by 0.84-fold; $P = .59$). However, *Pgr* mRNA expression was greatly decreased by 12.44-fold ($P = .01$) compared

to the corresponding control group. Progesterone receptor gene expression was decreased to a far greater extent than other genes measured in the distal endometriosis model and more than in the proximal model.

Discussion

This is the first controlled experiment to describe a systemic effect of endometriosis. Endometriosis had an effect on the uterus even when located exclusively outside the peritoneal cavity. In general, the effect of remote disease was less profound than that of local disease in proximity to the uterus. An exception to this paradigm of effect varying directly with proximity was seen with *Pgr*. The most significant effect on uterine *Pgr* expression was seen with remote disease. Progesterone receptor may be more profoundly affected by systemic disease. Although the mechanism of the altered eutopic gene expression that has been widely reported in endometriosis is unknown, it must be mediated, at least in part, by systemic signals, and we speculate that the inflammatory response to lesions outside the peritoneal cavity may be greater and that systemic inflammation may have a significant effect on *Pgr*.

Progesterone resistance has been well documented in women with endometriosis. The decreased *Pgr* gene expression may be tied to progesterone resistance that is a hallmark of more severe disease. Diminished *Pgr* is induced systemically and is predicted to allow lesion growth, escape from therapy as well as decrease endometrial receptivity. The systemic reduction in *Pgr* may allow lesions outside the uterus to escape progesterone-induced differentiation and allow the propagation of the disease.

Multiple mechanisms likely contribute to the altered gene expression seen in the eutopic endometrium of women with endometriosis. The systemic effect may be due to chronic inflammation as has been described in women with endometriosis.⁵² Alterations in immune function and production of inflammatory modulators can have an effect far from the lesions. Similarly, we have reported an increase in circulating microRNAs in women with endometriosis.⁵³⁻⁵⁵ MicroRNAs can regulate gene expression in organs remote from the site of production. In addition to classic inflammatory modulators, microRNAs are a likely mediator of the distant effects of endometriosis.

We have previously demonstrated that bone marrow-derived cells contribute to regeneration of the endometrium.⁸⁻¹¹ Endometriosis competes with the uterus for the very limited supply of circulating stem cells, thus restricting their availability to engraft the uterus.^{12,56,57} Remote lesions will act as a stem cell sponge, attracting circulating stem cells and preventing their incorporation in the eutopic endometrium. The loss of stem cells prevents cellular repair of the endometrium and may contribute to altered gene expression.

Alternatively, cell trafficking from the lesions to the uterus may alter receptivity. We have also shown that cells from lesions of endometriosis are able to migrate through the circulation to the endometrium.⁵⁸ These cells undergo a mesenchymal

to epithelial transition, however, they are not consistently incorporated in uterine glands or surface epithelium. Incorporation of epithelial cells in the uterine stroma interferes with the normal paracrine epithelial–stromal communication, altering uterine gene expression. Endometriosis, independent of location, prevents new stem cell incorporation to the uterus and acts as a source of cells that are improperly incorporated into the endometrium.

These findings have clinical implications. These data demonstrate that endometriosis is a systemic disease. Proximity to the uterus is not required to elicit all of the deleterious effects on the uterus. Some effects are direct, and there were several genes whose expression was changed only when the endometriosis was near the uterus.^{32,59–65} Clearly, remote endometriosis had an effect on uterine gene expression as well. The remote effects were fewer and specific. The decreased expression of *Hoxa10*, *Hoxa11*, *Igfbp1*, and *Klf9* in mice with proximal lesions was mostly in agreement with the results previously reported in murine endometriosis model by Lee et al.²⁶ However, we observed decreased expression of *Pgr* in contrast to the model by Lee et al in which *PR-B* expression was increased while *PR-A* was decreased. This discordant results with respect to *Pgr* expression between murine endometriosis models likely result from differences in the timing of tissue collection, however, we suggest that in both models, there is decreased *PR-A* expression. *PR-A* expression is the functional form of *PR* in the uterus and accounts for the decrease in both model systems. The correlation in the decrease of *Klf9* and *Pgr* expression is in agreement with the results reported by Heard et al⁶⁶ and Pabona et al.⁶⁴ Future studies will include analysis of protein levels of *PR* splice variants as well as examination of human samples from women with extraperitoneal endometriosis.

Endometriosis has an effect on endometrial receptivity independent of location. Although we measured an uterine effect, endometriosis may affect other organs as well. Women with endometriosis often present with diffuse systemic symptoms. The lesions can affect remote organs that include the uterus and likely other tissues as well. The mechanism of this effect is not fully known, however, endometriosis has been described as an inflammatory condition as well as a stem cell disease. Endometriosis, perhaps through multiple mechanisms, likely has an effect on multiple organ systems and truly should be classified as a systemic disease. The nonpelvic manifestations of the disease are likely caused by the systemic inflammation or direct cell trafficking to remote organs.

In summary, we demonstrated that the aberrant expression of selected endometrial receptivity markers including *Hoxa10*, *Hoxa11*, *Igfbp1*, *Klf9*, and *Pgr* is influenced by the presence of endometriosis. Many of these genes were profoundly affected by disease located in the peritoneal cavity, suggesting a local and direct effect on endometrial receptivity. We also note a specific effect of disease located far from the uterus and the peritoneal cavity, demonstrating that endometriosis is also a systemic disease. Local and systemic effects of the disease both likely contribute to the clinical manifestations of endometriosis.

Future studies will further explore the mediators of these distinct effects. Understanding the cellular molecular mechanisms that are influencing systemic alterations may allow for a better understanding of the totality of this disease as well as allow for novel targeted therapies.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by U54 HD052668 from the National Institutes of Health (NIH).

References

- Giudice LC, Kao LC. Endometriosis. *Lancet*. 2004;364(9447):1789–1799.
- Bulun SE. Endometriosis. *N Engl J Med*. 2009;360(3):268–279.
- Macer ML, Taylor HS. Endometriosis and infertility: a review of the pathogenesis and treatment of endometriosis-associated infertility. *Obstet Gynecol Clin North Am*. 2012;39(4):535–549.
- Sampson J. Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol*. 1927;14(1):422–469.
- Gruenewald P. Origin of endometriosis from the mesenchyme of the celomic walls. *Am J Obs Gynecol*. 1942;44(3):470–474.
- Bruner-Tran KL, Herington JL, Duleba AJ, Taylor HS, Osteen KG. Medical management of endometriosis: emerging evidence linking inflammation to disease pathophysiology. *Minerva Ginecol*. 2013;65(2):199–213.
- Taylor HS, Osteen KG, Bruner-Tran KL, et al. Novel therapies targeting endometriosis. *Reprod Sci*. 2011;18(9):814–823.
- Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *JAMA*. 2004;292(1):81–85.
- Du H, Taylor HS. Contribution of bone marrow-derived stem cells to endometrium and endometriosis. *Stem Cells*. 2007;25(8):2082–2086.
- Sasson IE, Taylor HS. Stem cells and the pathogenesis of endometriosis. *Ann N Y Acad Sci*. 2008;1127:106–115.
- Figueira PG, Abrão MS, Krikun G, Taylor HS. Stem cells in endometrium and their role in the pathogenesis of endometriosis. *Ann N Y Acad Sci*. 2011;1221:10–17.
- Wang X, Mamillapalli R, Mutlu L, Du H, Taylor HS. Chemoattraction of bone marrow-derived stem cells towards human endometrial stromal cells is mediated by estradiol regulated CXCL12 and CXCR4 expression. *Stem Cell Res*. 2015;15(1):14–22.
- Kinugasa S, Shinohara K, Wakatsuki A. Increased asymmetric dimethylarginine and enhanced inflammation are associated with impaired vascular reactivity in women with endometriosis. *Atherosclerosis*. 2011;219(2):784–788.
- Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol*. 2014;10(5):261–275.

15. Riley CF, Moen MH, Videm V. Inflammatory markers in endometriosis: reduced peritoneal neutrophil response in minimal endometriosis. *Acta Obstet Gynecol Scand.* 2007;86(7):877-881.
16. Agic A, Xu H, Finas D, Banz C, Diedrich K, Hornung D. Is endometriosis associated with systemic subclinical inflammation? *Gynecol Obstet Invest.* 2006;62(3):139-147.
17. Ness RB, Modugno F. Endometriosis as a model for inflammation-hormone interactions in ovarian and breast cancers. *Eur J Cancer.* 2006;42(6):691-703.
18. Berkkanoglu M, Arici A. Immunology and endometriosis. *Am J Reprod Immunol.* 2003;50(1):48-59.
19. Berbic M, Fraser IS. Immunology of normal and abnormal menstruation. *Womens Health (Lond Engl).* 2013;9(4):387-395.
20. Malutan AM, Drugan T, Costin N, et al. Pro-inflammatory cytokines for evaluation of inflammatory status in endometriosis. *Cent Eur J Immunol.* 2015;40(1):96-102.
21. Kocbek V, Vouk K, Bersinger NA, Mueller MD, Lanišnik Rižner T. Panels of cytokines and other secretory proteins as potential biomarkers of ovarian endometriosis. *J Mol Diagn.* 2015;17(3):325-334.
22. Králíčková M, Vetvicka V. Immunological aspects of endometriosis: a review. *Ann Transl Med.* 2015;3(11):153.
23. Cakmak H, Taylor HS. Implantation failure: molecular mechanisms and clinical treatment. *Hum Reprod Update.* 2011;17(2):242-253.
24. Cakmak H, Taylor HS. Molecular mechanisms of treatment resistance in endometriosis: the role of progesterone-hox gene interactions. *Semin Reprod Med.* 2010;28(1):69-74.
25. Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajcir N, Agarwal A. Pathogenic mechanisms in endometriosis-associated infertility. *Fertil Steril.* 2008;90(2):247-257.
26. Lee B, Du H, Taylor HS. Experimental murine endometriosis induces DNA methylation and altered gene expression in eutopic endometrium. *Biol Reprod.* 2009;80(1):79-85.
27. Giudice LC. Genes associated with embryonic attachment and implantation and the role of progesterone. *J Reprod Med.* 1999;44(2):165-171.
28. Rackow BW, Taylor HS. Submucosal uterine leiomyomas have a global effect on molecular determinants of endometrial receptivity. *Fertil Steril.* 2010;93(6):2027-2034.
29. Doherty LF, Taylor HS. Leiomyoma-derived transforming growth factor- β impairs bone morphogenetic protein-2-mediated endometrial receptivity. *Fertil Steril.* 2015;103(3):845-852.
30. Rackow BW, Jorgensen E, Taylor HS. Endometrial polyps affect uterine receptivity. *Fertil Steril.* 2011;95(8):2690-2692.
31. Franasiak JM, Holoch KJ, Yuan L, Schammel DP, Young SL, Lessey BA. Prospective assessment of midsecretory endometrial leukemia inhibitor factor expression versus $\alpha\beta 3$ testing in women with unexplained infertility. *Fertil Steril.* 2014;101(6):1724-1731.
32. Naqvi H, Ilagan Y, Krikun G, Taylor HS. Altered genome-wide methylation in endometriosis. *Reprod Sci.* 2014;21(10):1237-1243.
33. Taylor HS, Vanden Heuvel GB, Igarashi P. A conserved Hox axis in the mouse and human female reproductive system: late establishment and persistent adult expression of the Hoxa cluster genes. *Biol Reprod.* 1997;57(6):1338-1345.
34. Akbas GE, Taylor HS. HOXC and HOXD gene expression in human endometrium: lack of redundancy with HOXA paralogs. *Biol Reprod.* 2004;70(1):39-45.
35. Taylor HS, Bagot C, Kardana A, Olive D, Arici A. HOX gene expression is altered in the endometrium of women with endometriosis. *Hum Reprod.* 1999;14(5):1328-1331.
36. Zanatta A, Rocha AM, Carvalho FM, et al. The role of the Hoxa10/HOXA10 gene in the etiology of endometriosis and its related infertility: a review. *J Assist Reprod Genet.* 2010;27(12):701-710.
37. Du H, Taylor HS. Molecular regulation of mullerian development by Hox genes. *Ann N Y Acad Sci.* 2004;1034:152-165.
38. Bagot CN, Troy PJ, Taylor HS. Alteration of maternal Hoxa10 expression by in vivo gene transfection affects implantation. *Gene Ther.* 2000;7(16):1378-1384.
39. Taylor HS, Igarashi P, Olive DL, Arici A. Sex steroids mediate HOXA11 expression in the human peri-implantation endometrium. *J Clin Endocrinol Metab.* 1999;84(3):1129-1135.
40. Taylor HS, Arici A, Olive D, Igarashi P. HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. *J Clin Invest.* 1998;101(7):1379-1384.
41. Eun Kwon H, Taylor HS. The role of HOX genes in human implantation. *Ann N Y Acad Sci.* 2004;1034:1-18.
42. Wu Y, Halverson G, Basir Z, Strawn E, Yan P, Guo S-W. Aberrant methylation at HOXA10 may be responsible for its aberrant expression in the endometrium of patients with endometriosis. *Am J Obstet Gynecol.* 2005;193(2):371-380.
43. Lydon JP, DeMayo FJ, Funk CR, et al. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev.* 1995;9(18):2266-2278.
44. Zhang X-L, Zhang D, Michel FJ, Blum JL, Simmen FA, Simmen RCM. Selective interactions of Kruppel-like factor 9/basic transcription element-binding protein with progesterone receptor isoforms A and B determine transcriptional activity of progesterone-responsive genes in endometrial epithelial cells. *J Biol Chem.* 2003;278(24):21474-21482.
45. Simmen RC, Eason RR, McQuown JR, et al. Subfertility, uterine hypoplasia, and partial progesterone resistance in mice lacking the Kruppel-like factor 9/basic transcription element-binding protein-1 (Bteb1) gene. *J Biol Chem.* 2004;279(28):29286-29294.
46. Du H, Sarno J, Taylor HS. HOXA10 inhibits Kruppel-like factor 9 expression in the human endometrial epithelium. *Biol Reprod.* 2010;83(2):205-211.
47. Nayak NR, Giudice LC. Comparative biology of the IGF system in endometrium, decidua, and placenta, and clinical implications for foetal growth and implantation disorders. *Placenta.* 2003;24(4):281-296.
48. Rutanen E-M. Insulin-like growth factors in endometrial function. *Gynecol Endocrinol.* 1998;12(6):399-406.
49. Ghazal S, McKinnon B, Zhou J, et al. H19 lncRNA alters stromal cell growth via IGF signaling in the endometrium of women with endometriosis. *EMBO Mol Med.* 2015;7(8):996-1003.
50. Kim H, Ku S-Y, Kim SH, Choi YM, Kim JG. Association between endometriosis and polymorphisms in insulin-like growth factor binding protein genes in Korean women. *Eur J Obstet Gynecol Reprod Biol.* 2012;162(1):96-101.

51. Kim JJ, Taylor HS, Lu Z, et al. Altered expression of HOXA10 in endometriosis: potential role in decidualization. *Mol Hum Reprod.* 2007;13(5):323-332.
52. Weiss G, Goldsmith LT, Taylor RN, Bellet D, Taylor HS. Inflammation in reproductive disorders. *Reprod Sci.* 2009;16(2):216-229.
53. Cho S, Mutlu L, Grechukhina O, Taylor HS. Circulating microRNAs as potential biomarkers for endometriosis. *Fertil Steril.* 2015;103(5):1252-1260.
54. Santamaria X, Taylor H. MicroRNA and gynecological reproductive diseases. *Fertil Steril.* 2014;101(6):1545-1551.
55. Petracco R, Grechukhina O, Popkhadze S, Massasa E, Zhou Y, Taylor HS. MicroRNA 135 regulates HOXA10 expression in endometriosis. *J Clin Endocrinol Metab.* 2011;96(12):e1925-e1933.
56. Sakr S, Naqvi H, Komm B, Taylor HS. Endometriosis impairs bone marrow-derived stem cell recruitment to the uterus whereas bazedoxifene treatment lead to endometriosis regression and improved uterine stem cell engraftment. *Endocrinology.* 2014;155(4):1489-1497.
57. Zhou Y, Gan Y, Taylor HS. Cigarette smoke inhibits recruitment of bone-marrow-derived stem cells to the uterus. *Reprod Toxicol.* 2011;31(2):123-127.
58. Santamaria X, Massasa EE, Taylor HS. Migration of cells from experimental endometriosis to the uterine endometrium. *Endocrinology.* 2012;153(11):5566-5574.
59. Cruz CD, Del Puerto HL, Rocha AL, et al. Expression of Nodal, Cripto, SMAD3, phosphorylated SMAD3, and SMAD4 in the proliferative endometrium of women with endometriosis. *Reprod Sci.* 2015;22(5):527-533.
60. Governini L, Carrarelli P, Rocha AL, et al. FOXL2 in human endometrium: hyperexpressed in endometriosis. *Reprod Sci.* 2014;21(10):1249-1255.
61. Lessey BA, Lebovic DI, Taylor RN. Eutopic endometrium in women with endometriosis: ground zero for the study of implantation defects. *Semin Reprod Med.* 2013;31(2):109-124.
62. Afshar Y, Hastings J, Roqueiro D, Jeong JW, Giudice LC, Fazleabas AT. Changes in eutopic endometrial gene expression during the progression of experimental endometriosis in the baboon, *Papio anubis*. *Biol Reprod.* 2013;88(2):44.
63. Kim JJ, Fazleabas AT. Uterine receptivity and implantation: the regulation and action of insulin-like growth factor binding protein-1 (IGFBP-1), HOXA10 and forkhead transcription factor-1 (FOXO-1) in the baboon endometrium. *Reprod Biol Endocrinol.* 2004;2:34.1-6.
64. Pabona JM, Simmen FA, Nikiforov MA, et al. Krüppel-like factor 9 and progesterone receptor coregulation of decidualizing endometrial stromal cells: implications for the pathogenesis of endometriosis. *J Clin Endocrinol Metab.* 2012;97(3):e376-e392.
65. Aghajanova L, Giudice LC. Molecular evidence for differences in endometrium in severe versus mild endometriosis. *Reprod Sci.* 2011;18(3):229-251.
66. Heard ME, Velarde MC, Giudice LC, Simmen FA, Simmen RC. Krüppel-like factor 13 deficiency in uterine endometrial cells contributes to defective steroid hormone receptor signaling but not lesion establishment in a mouse model of endometriosis. *Biol Reprod.* 2015;92(6):140.