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Review article

Epithelial-to-mesenchymal transition in the development of adenomyosis

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ABSTRACT

Adenomyosis is a hormone-related disease that affects 10–66% of women, and women with this disorder suffer from menorrhagia, dysmenorrhea, pelvic pain, abnormal uterine bleeding, and/or infertility. Regarding the etiology of the disease, the current trend of thought is that adenomyosis or adenomyoma results as a down-growth and invagination of the endometrial basalis into the adjacent myometrium after disruption of the normally intact boundary between the two. The eutopic endometrium of adenomyosis presents invasive characteristics, including increased angiogenesis and proliferation, decreased apoptosis, induction of the local production of estrogens, induction of progesterone resistance, and impaired cytokine expression, and these changes enhance the ability of the endometrium to infiltrate the junctional zone myometrium and the growth of ectopic tissue. Hysterectomy is the major strategy to relieve secondary dysmenorrhea caused by adenomyosis. However, fertility and uterine preservation are compromised by such treatment. The traditional pharmacological therapies for adenomyosis are primarily aimed at the suppression of endogenous estrogen production, but the results are not satisfactory. Thus, there is an urgent need to develop novel treatment strategies for adenomyosis. There has been evidence that indicates that the estrogen-induced epithelial–mesenchymal transition (EMT) may play a role in the development of adenomyosis. In this article, we will concentrate on the estrogen-induced EMT in the pathogenesis of adenomyosis.

Introduction

Adenomyosis is a common gynecologic disorder that affects 10–66% of women, and women with adenomyosis often present with menorrhagia, dysmenorrhea, pelvic pain, abnormal uterine bleeding, and/or infertility.[1] The disorder is defined as the presence of ectopic endometrial glandular and stromal cells located 2.5 mm below the endometrial-myometrial interface with surrounding myometrial hyperplasia and hypertrophy.[2–4] In the past, the term "adenomyoma" was used to describe this type of lesion. Until 1925, 2 years before Sampson[5] created the term "endometriosis" to represent the presence of uterine mucosa in the peritoneal cavity, Frankl[6] used the term "adenomyosis" to describe the direct connection of the endometrium with the islands of mucosa located in the musculature. Frankl[6] defined the term "adenomyosis uteri" and explained that "I have chosen the name of adenomyosis, which does not suggest any inflammatory genesis as do terms like adenometritis, adenomyositis, and adenomyometritis". In 1972, Bird[2] provided the current definition of adenomyosis as "adenomyosis may be defined as the benign invasion of endometrium into the myometrium, producing a diffusely enlarged uterus which microscopically exhibits ectopic non-neoplastic, endometrial glands and stroma surrounded by the hypertrophic and hyperplastic myometrium".[7]

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Hysterectomy is the major strategy to relieve secondary dysmenorrhea caused by adenomyosis. However, fertility and uterine preservation are compromised by such treatment. Removing adenomyotic lesions instead of hysterectomy is another strategy to treat adenomyosis,[8,9] however, the effect is temporary, and most women quickly develop adenomyosis and must undergo hysterectomy within 1 year. Even if a normal pregnancy is achieved after removing adenomyotic lesions, safety remains a major concern during pregnancy. Residual adenomyosis in the myometrium combined with the possible inhibition of pregnancy-related changes, such as uterine softening, may increase the risk of miscarriage or uterine rupture. Traditional pharmacological therapies for adenomyosis are primarily aimed at the suppression of endogenous estrogen production by the application of GnRH agonists and low-dose oral contraceptives.[10] However, the results are not satisfactory. Thus, there is an urgent need to develop novel treatment strategies for adenomyosis.[11]

The pathogenesis of adenomyosis still remains unclear, and there are several postulated mechanisms of pathogenesis: invagination of basal endometrium, local hyperestrogenism, and mechanical forces manifesting as hyper or dysperistalsis.[3,4,12–16] Recently, there have been several studies that proposed that the estrogen-induced epithelial–mesenchymal transition (EMT) may play a role in the development of adenomyosis.[17–19] EMT is a process by which epithelial cells lose their polarity and are converted to a mesenchymal phenotype that is crucial in embryogenesis, fibrosis, and tumor metastasis.[20] In this review, we will concentrate on the pathogenesis of adenomyosis, which is related to EMT.

Pathogenesis of adenomyosis: Invagination of the basal endometrium

The current definition of adenomyosis is the benign invasion of the endometrium into the myometrium, producing a diffusely enlarged uterus, which microscopically exhibits ectopic non-neoplastic, endometrial glands, and stroma surrounded by the hypertrophic and hyperplastic myometrium.[2,7] Brosens et al[21] demonstrated on a magnetic resonance image (MRI) that there were two distinct zones: the submucosal layer, also called the inner myometrium (IM) or junctional zone (JZ), and the outer myometrium (OM). The JZ is not only structurally different but also functionally different from the outer myometrium. The irregular thickening of the JZ has been postulated as the magnetic resonance criterion for the diagnosis of adenomyosis. Brosens et al[21] postulated that this magnetic resonance appearance relies on the disruption of the inner myometrial architecture secondary to smooth muscle hyperplasia. Adenomyosis can also be diagnosed by three-dimensional (3D) ultrasonography, which reveals irregular thickening of the JZ associated with adenomyosis. Disruption of the specific microenvironment in the basal endometrium may also explain the structural and functional abnormalities of the JZ, such as hyperperistalsis, dysperistalsis, and inordinate smooth muscle proliferation associated with endometriosis and adenomyosis.[22–24]

Several human and experimental studies proposed that adenomyosis occurs by invagination of the basal endometrium into the JZ.[25] On imaging, abnormal thickening of the subendometrial myometrium includes basal endometrium and the JZ. The JZ may represent a region of structural weakness and myometrial dysfunction of varying severity susceptible to an invagination of the stromal cells.[25] Following the invagination of stromal cells, invasion of glandular cells, abnormal growth, and differentiation, these cells are subsequently surrounded by hypertrophic and hyperplastic myometrium.[26]

These data suggest that adenomyosis may be caused by defects in the formation of the JZ of the uterus and that invagination of the endometrium may play a role in the pathogenesis of adenomyosis.

Chronic inflammation and angiogenesis in adenomyosis

Chronic inflammation plays a crucial role in the pathogenesis of adenomyosis, as the presence of ectopic lesions is associated with the overproduction of prostaglandins, cytokines, and chemokines.[3,27] Abundant macrophages infiltrating the ectopic lesion express typical manifestations of facilitating growth and promoting neuroangiogenesis. In the peritoneal fluid of patients with endometriosis, there is a unique protein, ENDO-1, which is similar to haptoglobin that can bind to the macrophages and reduce their phagocytic ability. Furthermore, after binding to ENDO-1, the macrophages produce more interleukin-6 (IL-6).[28] Other increased cytokines include macrophage migration inhibitory factor (MIF), tumor necrosis factor-α (TNF-α), IL-1β, IL-8, regulated on activation normal T expressed and secreted (RANTES), and monocyte chemotactic protein-1 (MCP-1). Among these cytokines, IL-8, RANTES, and MCP-1 also act as chemoattractants, which facilitate the recruitment of macrophages and cause subsequent abundant peritoneal cytokine accumulation (Fig. 1)[3,27,29]

Aberrant prostaglandin accumulation also plays a role in the disease pathogenesis as well as in the clinical manifestations of dysmenorrhea, chronic pelvic pain, and infertility. Peritoneal macrophages from women with endometriosis express higher levels of cyclo-oxygenase-2 (COX-2) and release significantly higher amounts of prostaglandins than macrophages from healthy women.[30] In the microenvironment at the ectopic lesions, TNF-α promotes endometrial cell production of prostaglandin F2α (PGF2α) and PGE2.[31] The IL-1β activation of COX-2 increases production of PGE2, which subsequently activates steroidogenic acute regulatory protein (StAR) and aromatase. Therefore, estrogen completes a positive feedback loop that induces the local hyperestrogenism by upregulating PGE2 synthesis. This pathway highlights the interplay of estrogen dependence and inflammation in the disease pathogenesis.[32,33] Moreover, the macrophage nuclear factor-kappa B (NF-κB)-dependent pathway is also involved in the pathogenesis and induces the subsequent transactivation of controlling angiogenesis and tissue remodeling.[34,35]

Increased invasiveness

Adenomyosis can be diagnosed by imaging studies with MRI and 3D ultrasonography, on which there is irregular thickening of the JZ and the so-called inner myometrium that is associated with adenomyosis.[22,23] Several human and experimental studies proposed that adenomyosis occurs by invagination of the basal endometrium into the inner layer of the IM known as the JZ.[25] On imaging, abnormal thickening of the subendometrial myometrium includes the basal endometrium and the JZ. The JZ may represent a region of structural weakness and myometrial dysfunction of varying severity that is susceptible to an invagination of the stromal cells.[25] Following invagination of the stromal cells, invasion of glandular cells, abnormal growth, and differentiation, these cells are subsequently surrounded by hypertrophic and hyperplastic myometrium.[26] These findings from imaging studies suggest that adenomyosis may be caused by defects in the formation of the JZ of the uterus.

Moreover, the increased invasiveness of the endometriotic cells lends weight to the basal endometrium invagination hypothesis.[36] The nonmalignant invasive endometriotic cells were identified as lacking the tumor suppressor molecule, E-cadherin, in contrast to the eutopic endometrium.[37] Benagiano and Brosens[38] suggested that the eutopic endometrium of adenomyosis presented more

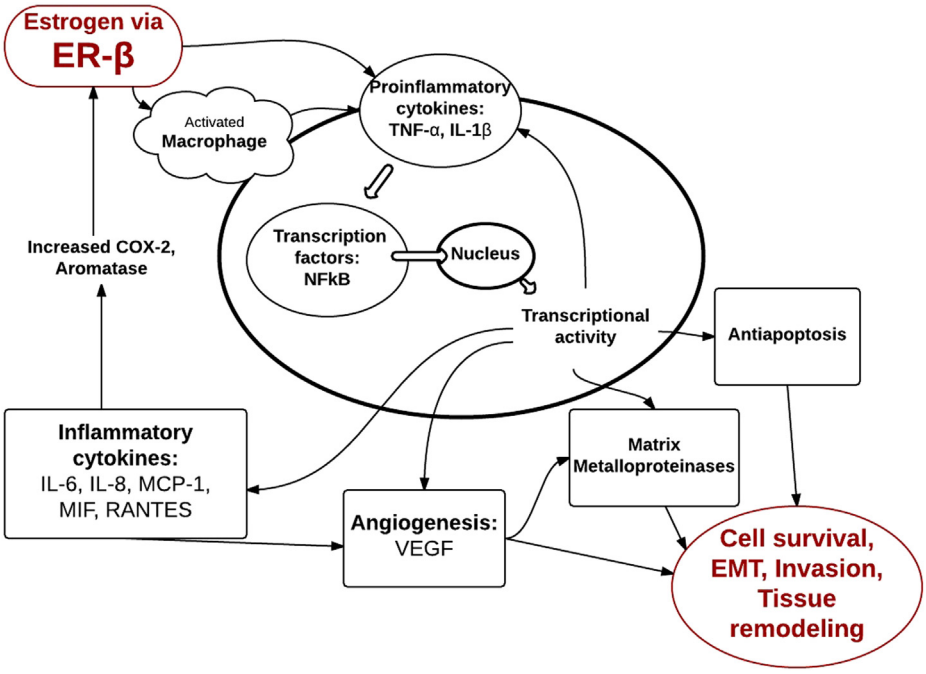

Fig. 1. A model of the most relevant molecular pathways involved in the pathophysiology of adenomyosis. COX-2 = cyclooxygenase-2;EMT = Epithelial–mesenchymal transition; IL = interleukin; NFκb = nuclear factor kappa b; MCP = monocyte chemotactic protein-1; MIF = macrophage migration inhibitory factor; RANTES = regulated on activation normal T expressed and secreted; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

invasive characteristics, including increased angiogenesis and proliferation, decreased apoptosis, induction of the local production of estrogens, induction of progesterone resistance, and impaired cytokine expression and that these changes enhanced the ability of the endometrium to infiltrate the JZ myometrium and the growth of ectopic tissue. *In vitro*, the adenomyotic stromal cells possessed greater invasiveness compared with normal endometrial stromal cells.[38] The invasiveness of normal endometrial stromal cells could be enhanced by the adenomyotic myocytes in a coculture model.[39] Thus, both stromal cells and myocytes may contribute to the pathogenesis of adenomyosis and reflect a whole uterine abnormality.[39]

Role of estrogen in the pathogenesis of adenomyosis

Estrogens influence many physiological processes and systems in mammals, including reproduction, the cardiovascular system, bone density, cognition, and behavior. Given this widespread role of estrogen in human physiology, it is not surprising that estrogen is also implicated in the development or progression of numerous diseases, which include but are not limited to various types of cancer (breast, ovarian, colorectal, prostate, and endometrial cancers), osteoporosis, leiomyoma, neurodegenerative diseases, cardiovascular disease, insulin resistance, lupus erythematosus, endometriosis, and obesity.[40] There are two main isoforms of estrogen receptors (ERs), ER-α and ER-β, which are encoded by separate genes, Esr1 and Esr2, respectively.

The pathogenesis of adenomyosis is considered an estrogen-related disorder, and a local rather than systemic hyperestrogenism may be implicated.[41] There is evidence that local hyperestrogenism results from the action of aromatase on androgen precursors[42]; aromatase mRNA is localized in adenomyotic tissue homogenate, and the aromatase protein is localized in the adenomyotic glands.[43] The aforementioned findings may be the reason that a higher estrogen level is detected in menstrual blood but not peripheral blood in women with adenomyosis.[44]

Moreover, the deficient methylation of the ER-β promoter results in the pathological overexpression of ER-β in endometriotic stromal cells. Thus, high levels of ER-β suppress ER-α expression, and a severely high ERβ-to-ERα ratio in endometriotic stromal cells is associated with decreased progesterone receptor (PR), particularly PR-B, and increased cyclo-oxygenase-2 (COX-2) levels, contributing to progesterone resistance and inflammation. Decreased PR expression may be associated with progesterone resistance.[45,46] In the normal endometrium, progesterone possesses an antiestrogen activity, in part by inducing 17β-hydroxysteroid dehydrogenase 2 (HSD17B2), which catalyzes the conversion of biologically potent estradiol (E2) to the less estrogenic estrone (E1).[47,48] Progesterone stimulates progesterone receptors in endometrial stromal cells to increase the formation of retinoic acid, which in turn induces HSD17B2 expression in endometrial epithelial cells in a paracrine fashion.[49] However, endometriotic stromal cells represent a limited response to progesterone and the production of retinoic acid. In endometriotic tissue, decreased retinoic acid levels lead to decreased epithelial HSD17B2 and the failure to suppress estradiol levels.[50] Thus, combined with high local estradiol production due to aberrant aromatase activity, this additional defect contributes to the abnormally high levels of estradiol in endometriotic tissue.

The expression levels of ER and PR isoforms during the menstrual cycle in eutopic and ectopic endometrium in women with adenomyosis were evaluated in an immunohistochemical study, and there was a significant decrease in ER-α expression during the midsecretory phase of the menstrual cycle in the adenomyotic functionalis glands and stroma. However, the expression of ER-α in the inner and outer myometrium was not significantly different in the adenomyotic functionalis glands and stroma. By comparison, the ER-β expression was significantly elevated in the adenomyotic functionalis gland during the proliferative phase and throughout the myometrium across the entire menstrual cycle.[51] Expression of PR-A is similar to that of PR-B, with reduced expression in the basalis stroma and the inner and outer myometrium in

adenomyosis. The pattern of ER-β, PR-A, and PR-B expression is similar in the endometrium basalis and adenomyotic foci. Higher ER-β expression and the lack of PR expression may be related to the pathogenesis of adenomyosis and provides evidence to explain the poor response of adenomyosis to progestational agents.[51]

EMT in adenomyosis

Epithelial cells are initiated to convert to migratory and invasive cells during the EMT. This process has been considered to be a fundamental event in morphogenesis, as it is intimately involved in the generation of tissues and organs during embryogenesis in both vertebrates and invertebrates. A similar process is represented during wound healing, a classic example of a process in adulthood in which EMT is crucial.[52] During EMT, epithelial cells lose their expression as epithelial markers (e.g., E-cadherin and plakoglobin), acquire mesenchymal marker expression (e.g., N-cadherin and vimentin), and gain increased migratory and invasive capabilities. Furthermore, several studies have shown that tumor cells may undergo EMT to facilitate cellular invasion, which can be stimulated either by extracellular cytokines, such as TGF-b, EGF, and FGF, or by intracellular cues, such as oncogenic Ras or NFkB signaling.[53] Then, EMT is initiated by a number of transcription factors that target the CDH1 gene promoter and repress the expression of E-cadherin.[54] These transcriptional repressors include Snail (Snail1), Snail2 (Slug), Twist1, Zeb1, Zeb2, Goosecoid, FOXC2, FoxQ1, KLF8, and Prrx. Thus, these EMT master regulators also induce tumor metastasis through inducing the cancer stem-cell phenotype, blocking oncogene-induced senescence and suppressing the host immune surveillance system.[20,53,54]

At an initial stage in cancer metastasis, tumor cells need to gain the ability to disseminate from a solid tumor mass and invade the surrounding stromal tissues, either as a group of cells (cohesive migration) or as single cells (mesenchymal invasion or amoeboid invasion).[55] This phenomenon has been confirmed in several *in vitro* cancer cell line studies.[56,57] Moreover, in the analysis of clinical patient samples, EMT among cancer cells in the invasive front of a number of different tumor types was demonstrated,[56,58,59] which may be crucial for tumor cell dissemination and metastasis.[58,60–62]

However, during the embryogenesis of the urogenital system, the endometrium is derived from intermediate mesoderm via the mesenchymal-to-epithelial transition (MET). By preserving some imprint of their mesenchymal origin, the endometrial epithelial cells may be particularly prone to return to this state, via EMT.[52] Previous studies have demonstrated that E-cadherin-negative epithelial cells were increased in peritoneal endometriosis compared with eutopic endometrium and that E-cadherin-negative, N-cadherin-positive endometriotic epithelial cells were invasive *in vitro*.[37,63]

Moreover, the tissue remodeling process may play a role in the pathogenesis of adenomyosis, and van Kaam et al[64] demonstrated that the presence of adenomyotic nodules in deeply infiltrating endometriosis lesions is accounted for by a reaction of the local environment to the presence of ectopic endometrium.[64] The tissue injury and repair (TIAR) theory was postulated by Leyendecker et al.[65] The wound healing process involves more extensive tissue remodeling through production of extracellular matrix (ECM) components, remodeling enzymes, cellular adhesion molecules, growth factors, cytokines, and chemokine genes. Activated macrophages generate various cytokines, including transforming growth factor (TGF)-β, which promotes tissue remodeling and subsequently causes fibroblasts to differentiate into myofibroblasts. Stromal cells were characterized as myofibroblasts due to their expression of α-smooth muscle actin, tropomyosin, desmin, and collagens.[66] Myofibroblasts play an important role in the development of adenomyosis by their expression of ECM proteins. These data suggest that myometrial hypertrophy is a response/reaction of the ectopic endometrial cells to the surrounding tissue, which shares characteristics with the physiological and pathological mechanisms of wound healing.

Furthermore, the extensive regulation, remodeling, and cyclical shedding of endometrial tissue requires the activation of matrix metalloproteinases (MMPs), which may contribute to the pathogenesis of adenomyosis. Gurates and Bulun[67] suggested that MMPs mediate endometrial breakdown and assist with new endometrium under the stimulation of estrogen. Most MMPs are under the influence of estrogen and progesterone: they are upregulated by estrogen and downregulated by progesterone.[68] Furthermore, compared with eutopic endometrium, ectopic endometrium possessed more tissue remodeling and endometrial invasion-involved activation of MMPs by extracellular matrix metalloproteinase inducer (EMMPRIN).[68] In one immunochemical analysis of uterine EMMPRIN, ER and PR expression throughout the menstrual cycle, the expression of EMMPRIN was strongest during the proliferative phase, while ER and PR were maximally expressed concomitantly.[69] Thus, the expression of EMMPRIN may be involved in the upregulation of MMPs, which enhances the endometrial invasive capability.

There is evidence that there is invasive behavior and cytoskeletal rearrangement in endometrial epithelial cells during endometriotic tissue implantation.[63] Compared with normal controls, E-cadherin is reduced in the uterine epithelial cells of women with endometriosis.[64] E-cadherin expression is further decreased in ectopic endometriotic lesions. The upregulation of vimentin expression was shown in endometriotic lesions compared with normal uterine endometrium.[70–72]

Estrogen-related EMT has been demonstrated through the upregulation of Snail or Slug in several tumor cell studies, such as in ovarian and breast cancers.[73] As a result, Chen et al[17] postulated that estrogen-induced EMT may be involved in the pathogenesis of adenomyosis, due to the invasive behavior and cytoskeletal rearrangement of endometrial epithelial cells and that the effect of estrogen-induced EMT could be abrogated by raloxifen, a selective estrogen receptor modulator. Zhou et al[18] demonstrated that through estrogen stimulation, there was upregulation of annexin A2, which was correlated with the expression of EMT markers in ectopic adenomyotic lesions and the severity of dysmenorrhea in women with adenomyosis. The upregulation of annexin-A2 expression could mediate phenotypic mesenchymal-like cellular changes, with structural and functional alterations in a β-catenin/T-cell factor (Tcf) signaling-associated manner, which could be reversed by the inhibition of annexin A2 expression. Moreover, annexin A2 enhanced the proangiogenic capacity of adenomyotic endometrial cells through the HIF-1α/VEGF-A pathway.[18] Oh et al[19] further proved that the β-catenin activation caused induction of Snail and ZEB1 expression, repression of E-cadherin expression, and induction of mesenchymal cell marker expression in endometrial epithelial cells during the pathogenesis of adenomyosis. In human adenomyotic lesions, CD10, a useful molecule of tumor invasion, was expressed compared with the negative result of normal controls. Moreover, in the adenomyotic mouse model with overexpression of β-catenin, the upregulation of COUP-TFII, which was associated with the induced extracellular matrix-degrading proteinases MMP2 and urokinase-type plasminogen activator (uPA), was noted in some cells.[19] MMP2 and uPA are known to play critical roles in angiogenesis and metastasis.[19] Thus, estrogen-induced tissue remodeling and EMT may be involved in the pathogenesis of adenomyosis.

There is conclusive evidence that estrogen, particularly through stimulating ER-β, upregulates tissue remodeling and the EMT of the basal endometrium, and these processes are also associated with local inflammatory processes and angiogenesis. Thus, ERβ-selective estradiol antagonists may provide a novel treatment for adenomyosis in the future.

Conclusion

Adenomyosis is considered a hormone-related disorder, and through estrogen stimulation, particularly ER-β, EMT is induced in the basal endometrium resulting in invagination into the myometrium surrounded by hypertrophic myometrium.

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References

1. Vercellini P, Vigano P, Somigliana E, Daguati R, Abbiati A, Fedele L. Adenomyosis: epidemiological factors. *Best Pract Res Clin Obstet Gynaecol*. 2006;20:465–477.
2. Bird CC, McElin TW, Manalo-Estrella P. The elusive adenomyosis of the uterus–revisited. *Am J Obstet Gynecol*. 1972;112:583–593.
3. Benagiano G, Habiba M, Brosens I. The pathophysiology of uterine adenomyosis: an update. *Fertil Steril*. 2012;98:572–579.
4. Ferenczy A. Pathophysiology of adenomyosis. *Hum Reprod Update*. 1998;4:312–322.
5. Sampson S. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol*. 1927;14:422–469.
6. Frankl O. Adenomyosis uteri. *Am J Obstet Gynecol*. 1925;10:680–684.
7. Benagiano G, Brosens I. History of adenomyosis. *Best Pract Res Clin Obstet Gynaecol*. 2006;20:449–463.
8. Hermann A, DeWilde RL. Laparascopic myomectomy-The gold standard. *Gynecol Minim Invasive Ther*. 2014;3:31–38.
9. Wong FWS, Lim DCE. Factors influencing the choice of hysterectomy approach for the management of fibroid uterus. *Gynecol Minim Invasive Ther*. 2013;2:61–64.
10. Guo SW. Keep the pressure on for more transparency of clinical trials on endometriosis. *Gynecol Minim Invasive Ther*. 2013;2:73–74.
11. Huang TS, Chen YJ, Chou TY, et al. Oestrogen–induced angiogenesis promotes adenomyosis by activating the Slug-VEGF axis in endometrial epithelial cells. *J Cell Mol Med*. 2014;18:1358–1371.
12. Curtis KM, Hillis SD, Marchbanks PA, Peterson HB. Disruption of the endometrial-myometrial border during pregnancy as a risk factor for adenomyosis. *Am J Obstet Gynecol*. 2002;187:543–544.
13. Mehasseb MK, Bell SC, Brown L, Pringle JH, Habiba M. Phenotypic characterisation of the inner and outer myometrium in normal and adenomyotic uteri. *Gynecol Obstet Invest*. 2011;71:217–224.
14. Mechsner S, Grum B, Gericke C, Loddenkemper C, Dudenhausen JW, Ebert AD. Possible roles of oxytocin receptor and vasopressin-1alpha receptor in the pathomechanism of dysperistalsis and dysmenorrhea in patients with adenomyosis uteri. *Fertil Steril*. 2010;94:2541–2546.
15. Parrott E, Butterworth M, Green A, White IN, Greaves P. Adenomyosis–a result of disordered stromal differentiation. *Am J Pathol*. 2001;159:623–630.
16. Shynlova O, Kwong R, Lye SJ. Mechanical stretch regulates hypertrophic phenotype of the myometrium during pregnancy. *Reproduction*. 2010;139:247–253.
17. Chen YJ, Li HY, Huang CH, et al. Oestrogen-induced epithelial-mesenchymal transition of endometrial epithelial cells contributes to the development of adenomyosis. *J Pathol*. 2010;222:261–270.
18. Zhou S, Yi T, Liu R, et al. Proteomics identification of annexin A2 as a key mediator in the metastasis and proangiogenesis of endometrial cells in human adenomyosis. *Mol Cell Proteomics*. 2012;11. M112 017988.
19. Oh SJ, Shin JH, Kim TH, et al. beta-Catenin activation contributes to the pathogenesis of adenomyosis through epithelial-mesenchymal transition. *J Pathol*. 2013;231:210–222.
20. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer*. 2009;9:265–273.
21. Brosens JJ, de Souza NM, Barker FG. Uterine junctional zone: function and disease. *Lancet*. 1995;346:558–560.
22. Naftalin J, Jurkovic D. The endometrial-myometrial junction: a fresh look at a busy crossing. *Ultrasound Obstet Gynecol*. 2009;34:1–11.
23. Hricak H, Alpers C, Crooks LE, Sheldon PE. Magnetic resonance imaging of the female pelvis: initial experience. *Am J Roentgenol*. 1983;141:1119–1128.
24. Leyendecker G, Kunz G, Wildt L, Beil D, Deininger H. Uterine hyperperistalsis and dysperistalsis as dysfunctions of the mechanism of rapid sperm transport in patients with endometriosis and infertility. *Hum Reprod*. 1996;11:1542–1551.
25. Benagiano G, Brosens I, Carrara S. Adenomyosis: new knowledge is generating new treatment strategies. *Womens Health*. 2009;5:297–311.
26. Hever A, Roth RB, Hevezi PA, et al. Molecular characterization of human adenomyosis. *Mol Hum Reprod*. 2006;12:737–748.
27. Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol*. 2014;10:261–275.
28. Sharpe-Timms KL, Piva M, Ricke EA, Surewicz K, Zhang YL, Zimmer RL. Endometriotic lesions synthesize and secrete a haptoglobin-like protein. *Biol Reprod*. 1998;58:988–994.
29. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril*. 2012;98:511–519.
30. Wu MH, Sun HS, Lin CC, et al. Distinct mechanisms regulate cyclooxygenase-1 and -2 in peritoneal macrophages of women with and without endometriosis. *Mol Hum Reprod*. 2002;8:1103–1110.
31. Chen DB, Yang ZM, Hilsenrath R, Le SP, Harper MJ. Stimulation of prostaglandin (PG) F2 alpha and PGE2 release by tumour necrosis factor-alpha and interleukin-1 alpha in cultured human luteal phase endometrial cells. *Hum Reprod*. 1995;10:2773–2780.
32. Attar E, Tokunaga H, Imir G, et al. Prostaglandin E2 via steroidogenic factor-1 coordinately regulates transcription of steroidogenic genes necessary for estrogen synthesis in endometriosis. *J Clin Endocrinol Metab*. 2009;94:623–631.
33. Bulun SE, Yang S, Fang Z, et al. Role of aromatase in endometrial disease. *J Steroid Biochem Mol Biol*. 2001;79:19–25.
34. Lousse JC, Van Langendonckt A, Gonzalez-Ramos R, Defrere S, Renkin E, Donnez J. Increased activation of nuclear factor-kappa B (NF-kappaB) in isolated peritoneal macrophages of patients with endometriosis. *Fertil Steril*. 2008;90:217–220.
35. Hagemann T, Lawrence T, McNeish I, et al. "Re-educating" tumor-associated macrophages by targeting NF-kappaB. *J Exp Med*. 2008;205:1261–1268.
36. Gaetje R, Kotzian S, Herrmann G, Baumann R, Starzinski-Powitz A. Invasiveness of endometriotic cells in vitro. *Lancet*. 1995;346:1463–1464.
37. Gaetje R, Kotzian S, Herrmann G, Baumann R, Starzinski-Powitz A. Nonmalignant epithelial cells, potentially invasive in human endometriosis, lack the tumor suppressor molecule E-cadherin. *Am J Pathol*. 1997;150:461–467.
38. Benagiano G, Brosens I. The endometrium in adenomyosis. *Womens Health*. 2012;8:301–312.
39. Mehasseb MK, Taylor AH, Pringle JH, Bell SC, Habiba M. Enhanced invasion of stromal cells from adenomyosis in a three-dimensional coculture model is augmented by the presence of myocytes from affected uteri. *Fertil Steril*. 2010;94:2547–2551.
40. Burns KA, Korach KS. Estrogen receptors and human disease: an update. *Arch Toxicol*. 2012;86:1491–1504.
41. Urabe M, Yamamoto T, Kitawaki J, Honjo H, Okada H. Estrogen biosynthesis in human uterine adenomyosis. *Acta Endocrinol*. 1989;121:259–264.
42. Ezaki K, Motoyama H, Sasaki H. Immunohistologic localization of estrone sulfatase in uterine endometrium and adenomyosis. *Obstet Gynecol*. 2001;98:815–819.
43. Kitawaki J, Noguchi T, Amatsu T, et al. Expression of aromatase cytochrome P450 protein and messenger ribonucleic acid in human endometriotic and adenomyotic tissues but not in normal endometrium. *Biol Reprod*. 1997;57:514–519.
44. Takahashi K, Nagata H, Kitao M. Clinical usefulness of determination of estradiol level in the menstrual blood for patients with endometriosis. *Nihon Sanka Fujinka Gakkai Zasshi*. 1989;41:1849–1850.
45. Bulun SE, Monsavais D, Pavone ME, et al. Role of estrogen receptor-beta in endometriosis. *Semin Reprod Med*. 2012;30:39–45.
46. Han SJ, Hawkins SM, Begum K, et al. A new isoform of steroid receptor coactivator-1 is crucial for pathogenic progression of endometriosis. *Nat Med*. 2012;18:1102–1111.
47. Yang S, Fang Z, Gurates B, et al. Stromal PRs mediate induction of 17beta-hydroxysteroid dehydrogenase type 2 expression in human endometrial epithelium: a paracrine mechanism for inactivation of E2. *Mol Endocrinol*. 2001;15:2093–2105.
48. Bulun SE, Cheng YH, Pavone ME, et al. Estrogen receptor-beta, estrogen receptor-alpha, and progesterone resistance in endometriosis. *Semin Reprod Med*. 2010;28:36–43.
49. Cheng YH, Yin P, Xue Q, Yilmaz B, Dawson MI, Bulun SE. Retinoic acid (RA) regulates 17beta-hydroxysteroid dehydrogenase type 2 expression in endometrium: interaction of RA receptors with specificity protein (SP) 1/SP3 for estradiol metabolism. *J Clin Endocrinol Metabol*. 2008;93:1915–1923.
50. Cheng YH, Imir A, Fenkci V, Yilmaz MB, Bulun SE. Stromal cells of endometriosis fail to produce paracrine factors that induce epithelial 17beta-hydroxysteroid dehydrogenase type 2 gene and its transcriptional regulator Sp1: a

mechanism for defective estradiol metabolism. *Am J Obstet Gynecol*. 2007;196, 391.e391–397; discussion 391.e397–398.
51. Mehasseb MK, Panchal R, Taylor AH, Brown L, Bell SC, Habiba M. Estrogen and progesterone receptor isoform distribution through the menstrual cycle in uteri with and without adenomyosis. *Fertil Steril*. 2011;95:2228–2235, 2235 e2221.
52. Nieto MA. The ins and outs of the epithelial to mesenchymal transition in health and disease. *Annu Rev Cell Dev Biol*. 2011;27:347–376.
53. Zheng H, Kang Y. Multilayer control of the EMT master regulators. *Oncogene*. 2014;33:1755–1763.
54. Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer*. 2007;7: 415–428.
55. Friedl P, Alexander S. Cancer invasion and the microenvironment: plasticity and reciprocity. *Cell*. 2011;147:992–1009.
56. Brabletz T, Jung A, Reu S, et al. Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc Natl Acad Sci USA*. 2001;98:10356–10361.
57. Tarin D, Thompson EW, Newgreen DF. The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res*. 2005;65:5996–6000. discussion 6000–6001.
58. Huang D, Du X. Crosstalk between tumor cells and microenvironment via Wnt pathway in colorectal cancer dissemination. *World J Gastroenterol*. 2008;14: 1823–1827.
59. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer*. 2002;2:442–454.
60. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell*. 2008;14:818–829.
61. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell*. 2009;139:871–890.
62. Matsuzaki S, Darcha C. Epithelial to mesenchymal transition-like and mesenchymal to epithelial transition-like processes might be involved in the pathogenesis of pelvic endometriosis. *Hum Reprod*. 2012;27:712–721.
63. Zeitvogel A, Baumann R, Starzinski-Powitz A. Identification of an invasive, N-cadherin-expressing epithelial cell type in endometriosis using a new cell culture model. *Am J Pathol*. 2001;159:1839–1852.
64. van Kaam KJ, Schouten JP, Nap AW, Dunselman GA, Groothuis PG. Fibromuscular differentiation in deeply infiltrating endometriosis is a reaction of resident fibroblasts to the presence of ectopic endometrium. *Hum Reprod*. 2008;23:2692–2700.
65. Leyendecker G, Wildt L, Mall G. The pathophysiology of endometriosis and adenomyosis: tissue injury and repair. *Arch Gynecol Obstet*. 2009;280:529–538.
66. Walter I, Handler J, Reifinger M, Aurich C. Association of endometriosis in horses with differentiation of periglandular myofibroblasts and changes of extracellular matrix proteins. *Reproduction*. 2001;121:581–586.
67. Gurates B, Bulun SE. Endometriosis: the ultimate hormonal disease. *Semin Reprod Med*. 2003;21:125–134.
68. Braundmeier AG, Fazleabas AT, Lessey BA, Guo H, Toole BP, Nowak RA. Extracellular matrix metalloproteinase inducer regulates metalloproteinases in human uterine endometrium. *J Clin Endocrinol Metabol*. 2006;91:2358–2365.
69. Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarty Jr KS. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J Clin Endocrinol Metabol*. 1988;67: 334–340.
70. Scotti S, Regidor PA, Schindler AE, Winterhager E. Reduced proliferation and cell adhesion in endometriosis. *Mol Hum Reprod*. 2000;6:610–617.
71. Ding JX, Feng YJ, Yao LQ, Yu M, Jin HY, Yin LH. The reinforcement of invasion in epithelial ovarian cancer cells by 17 beta-Estradiol is associated with up-regulation of Snail. *Gynecol Oncol*. 2006;103:623–630.
72. Huang Y, Fernandez SV, Goodwin S, et al. Epithelial to mesenchymal transition in human breast epithelial cells transformed by 17beta-estradiol. *Cancer Res*. 2007;67:11147–11157.
73. Park SH, Cheung LW, Wong AS, Leung PC. Estrogen regulates Snail and Slug in the down-regulation of E-cadherin and induces metastatic potential of ovarian cancer cells through estrogen receptor alpha. *Mol Endocrinol*. 2008;22: 2085–2098.