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.

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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. 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. In the past, the term “adenomyoma” was used to describe this type of lesion. Until 1925, 2 years before Sampson® created the term “endometriosis” to represent the presence of uterine mucosa in the peritoneal cavity, Frankl used the term “adenomyosis” to describe the direct connection of the endometrium with the islands of mucosa located in the musculature. Frankl® 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® 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”.2

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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.\textsuperscript{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.\textsuperscript{10} However, the results are not satisfactory. Thus, there is an urgent need to develop novel treatment strategies for adenomyosis.\textsuperscript{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.\textsuperscript{3,4,12\textendash16} 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.\textsuperscript{17\textendash19} 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.\textsuperscript{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.\textsuperscript{2,7} Brosens et al.\textsuperscript{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.\textsuperscript{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.\textsuperscript{3\textendash22}

Several human and experimental studies proposed that adenomyosis occurs by invagination of the basal endometrium into the JZ.\textsuperscript{23} 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.\textsuperscript{24} Following the invagination of stromal cells, invasion of glandular cells, abnormal growth, and differentiation, these cells are subsequently surrounded by hypertrophic and hyperplastic myometrium.\textsuperscript{25} 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.\textsuperscript{2,4,27} 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).\textsuperscript{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)\textsuperscript{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 cyclooxygenase-2 (COX-2) and release significantly higher amounts of prostaglandins than macrophages from healthy women.\textsuperscript{30} In the microenvironment at the ectopic lesions, TNF-α promotes endometrial cell production of prostaglandin F2α (PGF2α) and PGE2.\textsuperscript{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.\textsuperscript{32,33} Moreover, the macrophage nuclear factor-kappa B (NF-kB)-dependent pathway is also involved in the pathogenesis and induces the subsequent transactivation of controlling angiogenesis and tissue remodeling.\textsuperscript{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.\textsuperscript{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.\textsuperscript{22} 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{36} The nonmalignant invasive endometriotic cells were identified as lacking the tumor suppressor molecule, E-cadherin, in contrast to the eutopic endometrium.\textsuperscript{27} Benagiano and Brosens\textsuperscript{38} suggested that the eutopic endometrium of adenomyosis presented more
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. The invasiveness of normal endometrial stromal cells could be enhanced by the adenomyotic myocytes in a coculture model. Thus, both stromal cells and myocytes may contribute to the pathogenesis of adenomyosis and reflect a whole uterine abnormality.

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. 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. There is evidence that local hyperestrogenism results from the action of aromatase on androgen precursors; aromatase mRNA is localized in adenomyotic stromal tissue, and the aromatase protein is localized in the adenomyotic glands. 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.

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. 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). 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. 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. 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. 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...
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. 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-β, EGF, and FGF, or by intracellular cues, such as oncogenic Ras or NFκB signaling. Then, EMT is initiated by a number of transcription factors that target the CDH1 gene promoter and repress the expression of E-cadherin. 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.1,2,3,5,54

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). This phenomenon has been confirmed in several in vitro cancer cell line studies.5,57 Moreover, in the analysis of clinical patient samples, EMT among cancer cells in the invasive front of a number of different tumor types was demonstrated5,58,59 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.57,63

Moreover, the tissue remodeling process may play a role in the pathogenesis of adenomyosis, and van Kaam et al64 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 Leye-decker et al.55 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.65 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 Bulun67 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).69 In one immunohistochemical 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 concurrently.70 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 al74 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 al75 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.76 Oh et al77 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, CD16, a useful molecule of tumor invasion, was expressed compared with the negative result of normal controls. Moreover, in the adenomyotic mouse model with over-expression of β-catenin, the upregulation of COUP-TFI, which was associated with the induced extracellular matrix-degrading proteinases MMP2 and urokinase-type plasminogen activator (uPA), was noted in some cells.78 MMP2 and uPA are known to play critical roles in angiogenesis and metastasis.79 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, ER-β-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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