Molecular Pathology and Therapy of Endometriosis: Revisited

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Abstract

Endometriosis is among one of the most challenging diseases of the 21st century that affects women in their reproductive age, and its etiology and pathogenesis remains enigmatic. Unravelling the molecular mechanisms that play a role in the etiopathogenesis of endometriosis has been the subject of intense investigation over the last decade. It is a complex hormone-dependent and multifactorial disease involving intricate communications of genetic, immunological, hormonal and environmental factors and the clear understanding of the etiopathogenesis of endometriosis is scarce, as indicated by many studies and recent setbacks in clinical trials. However, the past few years have witnessed the emergence and discovery of new molecular mechanisms underlying the progression of endometriosis and these new leads give hope for better therapies. After a systematic review of the literature, we report the current findings and mechanisms on the progression of this disease with an emphasis on understanding advances in the molecular pathology that may lead to new therapies. Understanding this disease using a multidisciplinary approach may ultimately provide an effective cure for this challenging disease.

Keywords
Pathogenesis; Angiogenesis; Autoimmunity; Estradiol; Clinical trials; Endometriosis

Introduction

Even though endometriosis was described 150 years ago it still remains an enigmatic disease [1]. This multi-faceted hormone (estradiol) dependent condition is characterized by the presence and growth of endometrial glands and stroma outside the uterine cavity [2]. It is a heritable disease with a recurrence risk of 5-7% for a first degree relative of an affected individual [3]. Ectopic endometrial tissue is commonly seen on the ovaries, pelvic peritoneum, rectovaginal septum, fallopian tubes, vagina, cervix and uterosacral ligaments and very rarely in the pleura and the brain. Some of the different anatomical sites of endometriosis are categorized as ovarian, peritoneal and deep-infiltrative endometriosis [4]. It is one of the most common gynaecological diseases, affecting up to 10% of all premenopausal women, and this occurrence increases in women with infertility, pelvic pain, or both, yet has an unknown etiopathogenesis and pathophysiology [2]. Major symptoms of endometriosis include severe pain and infertility that considerably decrease the quality of life in premenopausal women [5]. Modern treatment modalities are medical, surgical or a combination of both. However, in surgical therapy, reports of recurrence are as high as 30% in patients followed three years post-laporoscopic surgery and 40-50% in patients five years after surgery [4,6-8]. Repeated surgeries for management of endometriosis are associated with increased morbidity and health care costs and damage to the ovarian reserve [4,9,10]. In this paper, considering the recent advances, we focus on the emerging themes on etiopathogenesis and therapy of endometriosis.

Pathogenesis of endometriosis

Theories on etiology and pathogenesis: The etiology and pathogenesis of endometriosis are not completely understood. There are many studies investigating the different cellular and molecular mechanisms involved in endometriosis. Theories attribute endometriosis to coelomic metaplasia, retrograde menstruation, lymphatic dissemination, vascular metastasis and cellular induction [11-17]. There is evidence that dysfunction of the uterus plays a crucial role [18]. In the menstrual effluent of women with endometriosis, fragments of basal endometrium have been identified. Findings by Leyendecker et al., concluded that pelvic endometriosis may result from transubial dislocation of fragments of basal endometrium [18]. Hyper- and dysperistalsis (peristalsis in the uterus directs transportation of the sperm) are also associated with endometriosis [19]. Oxidative stress is among one of the factors that play a role in the pathogenesis of endometriosis. Oxidative stress in peritoneal environment results from elevated free radicals and lowered antioxidant potential and the cellular responses to oxidative stress is also determined by the molecular and genetic changes in the tissues. In this regard, Lambrinoudaki and co-workers demonstrated that several markers of oxidative stress are elevated in the serum of women with endometriosis, suggesting a systemic phenomenon [20]. Furthermore, it has been shown that vitamin E binding protein afamin, differed in absolute quantity in peritoneal fluid of women with endometriosis when compared to normal women [21]. These results suggest a role of oxidative stress in the pathogenesis of endometriosis and antioxidant supplementation may be useful in the treatment. Endocannabinoid system consists of bioactive lipids that activate the cannabinoid receptors (CB1 and CB2). The components of endocannabinoid system (CB1, CB2 receptors are expressed and differentially regulated throughout the menstrual cycle in the endometrium [22]. Selective receptor agonists of the endocannabinoid system (WIN 55212-2) have been shown to decrease cell proliferation and control pain symptoms [22]. Few other studies have suggested the involvement of CB1 and the potential role for endocannabinoid system in the abnormal growth and pain in endometriosis [22]. These studies seem to suggest that these may be targets for therapy [22]. Human chorionic gonadotropin, an embryo-derived signal regulates gene expression in the endometrium which modulates embryo attachment, extracellular matrix remodelling [23]. Studies in the baboon model of endometriosis have shown blunt molecular response in eutopic endometrium to human chorionic gonadotropin, which may explain implantation failure and infertility in women with...
endometriosis [23]. Most of the recent findings of the pathogenesis of endometriosis are represented as a model in figure 1.

Angiogenesis in the maintenance of endometriosis: An effective blood supply is essential for the continued existence of endometrial implants for the development of endometriosis and many studies have focussed on mechanisms regulating angiogenesis [24]. Angiogenesis may occur by at least four mechanisms including: sprouting, intussusceptions, elongation/widening or incorporation of circulating endothelial cells into endometrial vessels [25,26]. Newly formed endometriotic lesions require nutrients and growth factors and hence, neovascularisation play a role in their development and persistence [24]. The angiogenic process in endometriosis share many common markers with tumour angiogenesis [24]. Endometrial growth and differentiation are controlled by steroids (estrogen and progesterone) at different phases during the menstrual cycle but the molecular mechanisms that are regulated by these steroids associated with angiogenesis are not clear. Most investigations on endometrial angiogenesis have focused on the effects of estrogens and vascular endothelial growth factor (VEGF). More recent studies have identified a role for tissue factor (TF) [27]. TF is a cellular receptor that plays a major role in the process of blood coagulation and also known to be involved in angiogenesis [27]. Immuno-histochemical studies have demonstrated an aberrant TF expression pattern in eutopic or ectopic endometrium obtained from women with endometriosis [27]. Cells present in the ectopic endometrium and involved in the immune responses showed the highest expression of TF [27]. Furthermore, the receptor activated by TF is also found to be up-regulated in the glandular epithelium of eutopic endometrium [27]. Very few studies have examined the vasculature of peritoneal endometriotic lesions [28,29]. Ovarian endometriotic lesions are heavily vascularised and blood vessels supplying the endometriotic lesions are free from pericytes [30]. This study also showed that a large number of blood vessels supplying the endometrial explants in women are immature and that antibodies against VEGF-A are effective in treatment of endometriosis by disrupting the angiogenic process [30]. Deep infiltrating endometriosis in the rectovaginal fossa is also well vascularised [31]. Various ex vivo and in vitro studies have been developed to study the angiogenic process involved in endometriotic lesion formation and it has been suggested that the imbalance between the pro- and anti-angiogenic growth factors in peritoneal fluid can trigger endometriosis [32]. Inflammatory cells such as macrophages contribute to the pro-angiogenic milieu [24]. Recent studies in the murine model have shown infiltration of dendritic cells to the sites of angiogenesis and promote the endometriotic lesion growth [33]. Although many angiogenic factors are associated with endometriosis, the mechanisms underlying revascularization of endometriotic lesions remain poorly understood. Some of the promising antiangiogenic drugs for the treatment for endometriosis include the antiangiogenic agent TNP-470 [34] capostat [35] angiogenesis [36] VEGF-A blocking antibodies [30] flt-1 decoy receptor [30] endostatin [35] 2 methoxyxestradiol [36] and selective cyclooxygenase inhibitors as reviewed by Van Langendonckt et al. [24]. Various aspects of merits and limitations of vascular therapy in the treatment of endometriosis has been reviewed elsewhere [24]. All the above studies indicate that antiangiogenic therapy may be useful in the management of endometriosis, however clinical evidence is still lacking.

Endometriosis as an estrogen-dependent disease: Suppression of estrogen leads to the regression of endometriotic lesions, and relapse occurs with the discontinuation of therapies that suppress estradiol production [37]. These findings suggest that endometriosis is dependent on estradiol for growth [37]. The successful treatment of endometriosis using aromatase inhibitors further supports the estrogen dependent nature of endometriosis [37]. Ablation expression of several estrogen-metabolizing enzymes has been reported in eutopic endometrium [2]. Increased expression of aromatase both at the mRNA and protein levels have been detected in the eutopic endometrium of patients with ovarian, peritoneal and deep infiltrating endometriosis and this expression did not differ throughout the menstrual cycle [38-40]. Estradiol metabolizing enzymes like 17β hydroxysteroid dehydrogenase (HSD) type 2 expressions have also been shown to be deficient in endometriotic tissue [24]. Receptors for estradiol (ERα and ERβ) have been detected in both eutopic and ecytopic endometrium. In the ecytopic endometrium, the expression of ERα is lower in patients with peritoneal and ovarian endometriosis [39]. ERβ was up regulated in the endometriotic tissue in women with ovarian endometriosis [40]. This upregulated ERβ could modulate the cell cycle and contribute to the proliferation of endometriotic stromal cells [41]. Estrogen metabolism and its role in endometriosis have been reviewed by Rizner recently [11]. Polymorphisms of ERβ (+1730 G/A polymorphism) can be associated with increased risk of infertility and endometriosis-associated infertility [42]. Recently it was shown by Kim et al. that the low-dose estrogen only regimen decreased uterine bleeding given as a short-term add-back therapy during post-operative GnRH agonist treatment after surgery for endometriosis [43]. In contrast to the peripheral blood, estradiol levels are elevated in menstrual blood of women with endometriosis where P450 aromatase is elevated in the ecytopic and eutopic...
endometrium [44]. CyR61, a target gene of estradiol is also found to be up-regulated in eutopic endometrium in women with endometrial migration, endometriosis, ectopic lesions and also in an experimental endometriosis model [45]. CyR61 gene codes for a secreted, cysteine-rich, heparin-binding protein that promotes cell adhesion, and neovascularisation [45]. Increased production in estradiol from these endometriotic lesions may result from impaired metabolism or the failure to metabolize estradiol due to progesterone resistance [46,47]. Steroidogenesis is also upregulated which is required for estrogen synthesis in endometriosis. The regulation of the steroidogenic genes are mediated by prostaglandin E2 via steroidogenic factor (SF-1) [48]. The tissue injury and repair (TIAR) model describes the pathogenesis of endometriosis in association with increased estrogen [48,49]. This model of TIAR has been described in detail elsewhere [50]. Interestingly, 2-methoxy estradiol biological active metabolite of estradiol is antiangiogenic and is a new target for the treatment of endometriosis [51]. Various changes associated with estrogen and prostaglandin (PG)E2 levels at different states of developing endometriosis disease are represented in figure 2.

**Endometriosis an epigenetic disease:** Many large-scale gene expression profiling studies have demonstrated the deregulation of several genes in endometriotic tissue over the past decade [52-55]. Hyper-methylation of HOXA10 gene was observed in endometrium from women with endometriosis [56]. This is the first evidence showing the importance of epigenetic modifications in endometriosis [4]. HOXA10 is expressed in the human endometrium, and its expression is increased during implantation with the rise in circulating progesterone concentrations. HOXA10 mediates progesterone responsiveness in the endometrium by regulating progesterone receptor cofactors such as the KLF9 [57]. There is a decrease in the expression of this gene in endometrium of women with endometriosis and associated infertility. Increased methylation decreases expression, and results in defective uterine receptivity as well as associated infertility with endometriosis. Another gene that is also hyper-methylated is the promoter of PR-B (progesterone receptor isoform B) [58]. This promoter methylation results in PR-B down regulation and progesterone resistance. DNMT1, DNMT3A and DNMT3B (DNA methyl transferases) are over expressed in endometriosis further suggesting that it is an epigenetic disease [59]. Over expression of these genes alters the methylation status of certain genes which results in an aberrant gene causing endometriosis. Steroidogenic factor (SF-1), a transcriptional factor essential for activation of multiple steroidogenic genes needed for estrogen biosynthesis is also altered in endometriotic stromal cells when compared to the normal endometrial stromal cells [60]. Increased methylation in this promoter region was observed for this gene in endometrial cells but not in immortalized endometriotic cells [60]. Endometriotic cell lines (immortalized) lack the intercellular adhesion protein E-cadherin (metastasis-suppressor protein) in epithelial tumor cells and this deregulation seems to be associated with invasiveness of endometriotic cells [4]. E-cadherin was found to be hyper methylated and treatment with histone deacetylase inhibitor, trichostatin A (TSA) resulted in reactivated expression in these endometriotic cell lines [61]. This reactivated expression is associated with decreased invasiveness which suggests that E-cadherin is silenced in the endometriotic tissue to allow for growth and invasion [61]. Recently, the presence of polymorphisms MMP-2 (-735 C/T) and MMP-9 (-1562 C/T) were associated with elevated risk for endometriosis and certain MMP-2 promoter haplotypes were more common than in control groups [62]. All these studies suggest that epigenetics play an important role in endometriosis also reviewed by Guo [4]. In this review, publications up to the end of June 2009 relating epigenetics and endometriosis were retrieved and reviewed and conclude that epigenetics (aberrant methylation and deregulation of miRNA) play an important role in the pathology of endometriosis and histone deacetylase inhibitors may be promising therapeutics for treating endometriosis [4].

**Animal models to study endometriosis:** Rodents and non-human primates such as the Japanese macaque, pigtailed macaque, rhesus monkeys and baboons have been used for the development of experimental endometriosis models and for medical therapy trials. Rodents are not good models to study as it lacks menstrual cycle like primates and endometriosis do not develop spontaneously in rodents. Some of the studies employing primate models for the therapeutic trials in endometriosis are discussed below. The primary target in therapeutic trials is the inflammatory cytokine tumor necrosis factor (TNF)-a which is a potent stimulator of the inflammatory process in endometriosis [63]. Anti-TNF therapy has shown to be a promising, non hormonal therapeutic option for the treatment of endometriosis [64]. Treatment with the anti-TNFa monoclonal antibody (cSN) post disease induction has been shown to reduce the formation of ectopic lesions [64]. Peroxisome proliferator-activated receptors (PPARs) have been shown to induce the regression of endometriotic explants in the rat and baboon model [65,66]. Recently a prospective, randomized, placebo-controlled study conducted in a baboon model has shown reduction in the initiation of endometriotic disease by PPAR-gamma ligand, pioglitazone [67]. This group of drugs abrogates the aberrant immune response and provides an anti-inflammatory milieu. Furthermore, the ligands for PPAR-gamma have been shown to regulate cell growth, apoptosis and angiogenesis in human endometrial cells [67]. These studies suggest that thiazolidinedione may be a promising medication in the treatment of endometriosis [67]. Treatment with antiangiogenic agents in animal models (both rodents and humans) for the effective treatment of endometriosis has been well studied [30].

**Autoimmunity and endometriosis:** Serum of patients with endometriosis-associated infertility has antibodies against carbonic anhydrase. Autoantibodies against this antigen have been observed.
in other autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis [68]. With the help of two dimensional electrophoresis, two proteins, alpha 2-Heremans Schmid glycoprotein (HS) and transferrin with molecular weight 64KDa and 72KDa which are bound to IgG were identified in patients with endometriosis when compared with controls [69]. The assay for antibodies against these proteins may be used for diagnosing endometriosis [69]. Laminin is a basement membrane protein which has function in embryogenesis, implantation and placentation. Auto antibodies to laminin-1 may be important in the development of autoimmune-mediated reproductive failures [70]. Expression of IgG anti-laminin-1 antibodies was involved in 90% of all endometriotic lesions found within infertile endometriosis patients [71,72]. Furthermore, increased levels of antibodies against laminin-1 were found in serum and peritoneal fluid of patients with stage II-III [73]. The frequency of protein tyrosine phosphatase N22 (PTPN22) polymorphism was identified in Italian [74], Polish [75] and Brazilian women [76] with and without a history of endometriosis and/or autoimmune diseases. In advanced stages of endometriosis, an allelic variation of the PTPN22 gene has been reported in Italian and Brazilian women [74,76]. A high frequency of endocrine and autoimmune disorders exists among women with endometriosis. Women with endometriosis have been found to have higher rates of hypothyroidism, fibromyalgia, chronic fatigue syndrome, autoimmune diseases, allergy or asthma [77]. Pelvic endometriosis may be related to the triad of alopecia universalis, autoimmune thyroiditis and multiple sclerosis as seen in Caucasian females [78]. There is also evidence for elevated cytokine production and increased IL-6 production in eutopic endometrium from women affected with endometriosis [79]. A recent study noted rheumatoid arthritis associated single nucleotide polymorphisms in endometriosis. There is an association of CCL21 (rs2812378) and HLA-DRB1 (rs660895) with moderate to severe endometriosis [80].

Treatment of endometriosis: Current medical therapy for endometriosis targets suppression of ovarian function, thereby reducing the effect of estrogen on ectopic endometrial implants. Currently, most treatment modalities only suppress the disease but cannot prevent it. Long-term therapy for chronic pelvic pain requires repeated courses of medical and/or surgical intervention. However, recurrence of pain is common within 6-12 months following treatment [81]. Pharmaceutical agents that are used to interfere with adhesion, invasion or persistence of the ectopic endometriotic lesions can also have detrimental effects on the development and implantation of the embryo. Manipulations of the hypothalamic-pituitary-ovarian axis to block ovarian estrogen secretion, via progestins (state of pseudopregnancy), locally inhibit the actions of estrogen on ectopic endometrium (treatment with progestins, androgenic progestins). These hormonal alterations allow for the management of endometriosis and pain relief [81-83]. Recently, statins, such as simvastatin, has been shown to induce apoptosis in human endometrial stromal cells [84]. Furthermore, resveratrol, a natural polyphenol with anti-proliferative and anti-inflammatory properties inhibited endometriosis in nude mice and also reduced invasion of human endometrial stromal cells in vitro [85]. Many encouraging preclinical studies for potential therapeutics against endometriosis have been reported within the last decade [86]. Among these studies many of them have undergone phase II/III clinical trials, but the results have yet to be published [86]. The diagnosis and management of endometriosis associated pain and infertility as per the guidelines from The American Society for Reproductive Medicine and the European Society of human reproduction and Embryology are reviewed in Giudice et al. [87]. Furthermore, the medical and surgical management for endometriosis related pelvic pain according to the FDA (Food and Drug administration) was reviewed recently by Giudice et al. [87]. This article discusses regarding the diagnosis and clinical staging of endometriosis and concludes with various recommendations in the management of pain and associated infertility in endometriosis [87]. Typically, empirical medical therapy is initiated before laparoscopic confirmation of endometriosis and it has been shown that non-steroidal anti-inflammatory drugs (NSAIDS) are commonly used to relieve dysmenorrhea by minimizing inflammation [87]. A combination of non-steroidal anti-inflammatory drugs with oral contraceptives can be used for treating endometriosis [87]. However, randomized, non-blinded trials have shown that medroxyprogesterone acetate is more effective in controlling pain when combined with oral contraceptives [88]. GnRH agonists can deplete pituitary gonadotropins and thereby interrupt the menstrual cycle resulting in a hypo-estrogenic state, leading to endometrial atrophy and amenorrhea [87]. A systematic review of 15 randomized trials involving 1,821 women showed improvement in pain scores (60-100%) for dysmenorrhea with the use of GnRH agonists [87,89]. Similar findings were observed with other medications such as Danazol, anti-progestins and combined oral contraceptives [87]. However, because GnRH agonists cause a hypo-estrogenic state, bone loss may occur and estrogen-progestagen add back therapies are generally recommended [87,90]. Endometriotic tissues express aromatase and produce local estrogens. As a result decreasing the ovarian estradiol alone may not completely control pain, and the use of aromatase inhibitors may provide additional benefits [87]. Aromatase inhibition has been shown to effectively reduce pain [91]. In a prospective study, use of letrozole, a non-steroidal competitive aromatase inhibitor caused ovarian cysts in patients [91]. Surgical therapy can be initiated as a first line treatment or after medical therapy fails [87]. Surgical procedures include excision, fulguration, or laser ablation of endometriotic implants on the peritoneum, excision, drainage, or ablation of endometriomas, resection of rectovaginal nodules, lysis of adhesions, and interruption of nerve pathways [87]. While ablation of a segment of the uterosacral ligament has not proved successful, randomized controlled trials have shown the superiority of laparoscopic ablation of endometriotic tissue combined with presacral neurectomy (removal of the nerve bundle within the boundaries of the inter-iliac triangle) over laparoscopic ablation alone in improving dysmenorrhea and reducing severe midline pain [87-92]. Postoperative medical therapy improves pain management in women with advanced disease (stage III or IV) with dysmenorrhea and non cyclic pelvic pain [87]. Postoperative treatment with GnRH agonists (>24 months) decreased the mean interval between surgery and symptom recurrence in patients when compared to others treated with placebo (12 months) [87,93].

Conclusions

Even though knowledge about endometriosis has increased, many unknowns still exist. Endometriosis is a multifactorial disease and involves hormonal, immunological and also epigenetic aberrancies. Recent literature has supported that these combined mechanisms contribute to its pathogenesis and pathophysiology. Endometriosis remains a major problem despite increasing knowledge regarding the cell biology of this disease. The outcomes of clinical trials directed at treatment are still not encouraging and remind us that more research
is needed to understand the biology of this disease. Especially, more studies should explore the differences in the molecular signatures of ectopic and eutopic endometrium which might determine the outcomes of the clinical trials. Since animal models differ in anatomy and physiology and may not actually represent human endometriosis disease, a proper establishment of animal model of endometriosis is needed in order to evaluate therapeutic alternatives. Multidisciplinary efforts involving basic, clinical and translational researchers are necessary to uncover the various factors involved in endometriosis and hopefully lead to improved treatment and management paradigms as well as disease prevention.

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