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Review

Pathogenesis of adenomyosis: an update on molecular mechanisms

Silvia Vannuccini [a], Claudia Tosti [a], Francisco Carmona [b], S Joseph Huang [c,d], Charles Chapron [e,f,g], Sun-Wei Guo [h,i], Felice Petraglia [a,*]

[a] *Obstetrics and Gynecology, Department of Molecular and Developmental Medicine University of Siena, Siena, Italy*
[b] *Clinical Institute of Gynecology, Obstetrics, and Neonatology, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain Group of Endocrinology, Gynecology and Human Reproduction, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain*
[c] *Department of Obstetrics and Gynecology, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan*
[d] *Department of Obstetrics and Gynecology, Morsasni College of Medicine, University of South Florida, Tampa, FL, USA*
[e] *Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Assistance Publique-Hôpitaux de Paris, Hôpital Universitaire Paris Centre, Centre Hospitalier Universitaire Cochin, Department of Gynaecology Obstetrics II and Reproductive Medicine, Paris, France*
[f] *Institut Cochin, INSERM U1016, Laboratoire d'Immunologie, Université Paris Descartes, Sorbonne Paris Cité, Paris, France*
[g] *Département de Génetique, Développement et Cancer, Université Paris Descartes, Sorbonne Paris Cité, Paris, France*
[h] *Shanghai OB/GYN Hospital, Fudan University, Shanghai 200011, China*
[i] *Shanghai Key Laboratory of Female Reproductive Endocrine-Related Diseases, Fudan University, Shanghai, China*

Felice Petraglia is Professor and Chairman of Obstetrics and Gynecology. He is Fellow ad Eundem of the Royal College of Obstetricians and Gynecologists (RCOG). He was President of the Society for Gynecologic Investigation (SGI). He is Editor-in-Chief of *Human Reproduction Update* and Section Head in Reproductive Endocrinology of Faculty 1000 Medicine.

KEY MESSAGE

Sex steroid receptors, proliferation and fibrosis, inflammatory mediators and neuroangiogenesis are key pathogenetic mediators of adenomyosis-related pain, abnormal uterine bleeding and infertility.

A B S T R A C T

Adenomyosis is a uterine disorder becoming more commonly diagnosed in women of reproductive age because of diagnostic imaging advancements. The new epidemiological scenario and the clinical evidence of pelvic pain, abnormal uterine bleeding and infertility are changing the classic perspective of adenomyosis as a premenopausal disease. In the last decade, the evaluation of multiple molecular mediators has improved our knowledge of pathogenic mechanisms of adenomyosis, supporting that this is an independent disease from endometriosis. Although they share common genetic mutations and epigenetic changes in sex steroid hormone receptors and similar inflammatory mediators, an increasing number of recent studies have shown pathogenic pathways specific for adenomyosis. A PubMed search up to October 2016 summarizes the key mediators of pain, abnormal uterine bleeding and infertility in adenomyosis, including sex steroid hormone receptors, inflammatory molecules, extracellular matrix enzymes, growth factors and neuroangiogenic factors.

* Corresponding author.
E-mail address: felice.petraglia@unisi.it (F Petraglia).

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Introduction

Adenomyosis is defined as the presence of ectopic endometrial glands and stroma surrounded by hyperplastic smooth muscle within the myometrium. It is a uterine disorder clinically presented with pelvic pain, abnormal uterine bleeding (AUB) and infertility. Dysmenorrhoea and dyspareunia are the most common symptoms; however, the clinical presentation of adenomyosis is often mixed and occasionally it may even be asymptomatic (Farquhar and Brosens, 2006). Rokitansky first recognized adenomyosis in 1860 but the term was first used by Frankl in 1925 (Benagiano et al., 2012; Leyendecker et al., 2006). Before the advancement of imaging techniques such as transvaginal ultrasound scan (TVUS) and magnetic resonance imaging (MRI), adenomyosis could only be diagnosed by histology after hysterectomy. Two different pathological aspects of adenomyosis are described: diffuse and focal forms (when a defined nodule is found, the term adenomyoma is also used). Adenomyosis and endometriosis share a number of features and it was found that, at least in some subgroups, the two conditions often coexist (Lazzeri et al., 2014; Li et al., 2014), so much so that for a long time adenomyosis had been termed endometriosis *interna*. Nevertheless, they are considered as two distinct entities because many differences have been observed in pathogenesis, risk factors and clinical presentation (Benagiano et al., 2014). Despite all these differences, the two conditions have many similarities in definition, symptomology and molecular aberrations (Li et al., 2013). Above all, adenomyosis and endometriosis share the same commonality of experiencing cyclic bleeding (Liu et al., 2016; Shen et al., 2016).

The pathogenic mechanisms of adenomyosis development are still debatable; however, sex steroid hormone aberrations, inflammation, altered cell proliferation and neuroangiogenesis are likely key pathogenic mechanisms of pain, AUB and infertility in adenomyosis. In this review, we summarize all the available evidence on specific pathways and mediators involved in the pathogenesis of adenomyosis, explaining the clinical presentation of the disease.

Sources

A PubMed search of the literature from 1950 to October 2016 was performed in order to summarize all evidence on pathogenic mechanisms of adenomyosis development and clinical presentation. All pertinent articles were examined and their reference lists were reviewed in order to identify other studies for potential inclusion. The literature search included the following terms: 'adenomyosis', 'adenomyoma', 'pathogenesis', 'pain', 'abnormal uterine bleeding', 'infertility', 'sex steroid hormones', 'sex steroid hormone receptors', 'inflammation', 'neoangiogenesis', 'growth factors', 'extracellular matrix (ECM)', 'fibrosis', 'proliferation' and 'neurogenic factors'. Only peer-reviewed, English-language journal articles were included.

Pathogenic hypotheses

Despite the prevalence of the disease, its precise aetiology and physiopathology remain in part unknown. Some hypotheses have been developed, suggesting the role played by the endometrium, the mechanism of tissue injury and repair (TIAR) and stem cell theory.

Invasion from the endometrium

According to the current theory, adenomyosis develops through downgrowth and invagination of the endometrium *basalis* into the myometrium through an altered or absent junctional zone (JZ) (Bergeron et al., 2006; Parrott et al., 2001). Thus, the endometrium can slip through bundles of weak smooth muscle fibres that have loosened their tissue cohesion. Dysregulation of genes and pathways in the eutopic endometrium may predispose to ectopic migration and implantation. The analysis of the global transcriptome of eutopic endometrial cells from women with clinically significant adenomyosis revealed 140 up-regulated and 884 down-regulated genes, compared with controls. Genes involved in regulation of apoptosis, steroid hormone responsiveness and extracellular matrix remodelling as well as microRNAs of unknown significance were found to be highly differentially expressed. Affected canonical pathways included eukaryotic initiation factor 2 (eIF2) signalling, oxidative phosphorylation, mitochondrial dysfunction, oestrogen receptor (ER) signalling, and mammalian target of rapamycin (mTOR) signalling (Herndon et al., 2016). These aberrant pathways may predispose toward the development, migration and survival of ectopic endometrial implants beyond the myometrial interface. However, further studies are needed to elucidate the biological significance of these aberrations, especially in the early developmental stage of the disease.

Mechanism of TIAR

The phenomenon of endometrial invasion may happen in a predisposed myometrium or in a traumatized endometrial–myometrial interface (Benagiano et al., 2012). Uterine auto-traumatization and initiation of the mechanism of TIAR have been considered as the primary events in the disease process. A condition of chronic proliferation and inflammation induced at the level of the archimetra by chronic uterine auto-traumatization supports one of the theories for adenomyosis pathophysiology (Leyendecker et al., 2015). Accordingly, the TIAR mechanism, in response to increased intrauterine pressure, may promote the migration of fragments of basal endometrium into the myometrium. In a recent study a new software was used to develop a conceptual two-dimensional model of the uterine wall subjected to a variety of intrauterine sinusoidal pressure waves with varying frequencies. It was noted that a decrease in wavelength and an increase in frequency of the subjected pressure wave led to high levels of stress near the inner uterine cavity. During menstruation, the highest stress was observed at the endometrial–myometrial interface. Hence, high stress caused by increased uterine activity may lead to tissue lesions and detachment of endometrial cells (Shaked et al., 2015).

Chronic peristaltic myometrial contractions induce microtrauma to the JZ, causing a vicious cycle in which local oestrogen production, COX-2 mediated (Chen et al., 2010a), promotes uterine peristalsis and further auto-traumatization (Gargett et al., 2016; Leyendecker et al., 2009).

On the basis of small-diameter nerve fibre proliferation observed in the myometrium of patients with chronic pelvic pain (Quinn and Kirk, 2002), Quinn postulated that nerve injury (denervation) in the uterus and/or uterosacral ligaments may lead to endometriosis (Quinn, 2004; Quinn and Kirk, 2004; Quinn, 2011) and also to adenomyosis (Quinn, 2007). Nerve injury might be due to either difficult intrapartum episodes or persistent straining to achieve defecation. The resulting re-innervation of the uterine isthmus may be the primary source of many pain symptoms in adenomyosis (Quinn, 2007).

While these hypotheses and theories are intuitive and attractive, the biggest challenge is to make them falsifiable, i.e. able to be tested with a well-designed experiment. In addition, these hypotheses and

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theories need to, by default, explain all existing data and should also predict new phenomena.

Stem cell theory

Another theory proposes that adenomyosis, like endometriosis, might develop through metaplasia from *de novo* ectopic intramyometrial endometrial tissue (Hufnagel et al., 2015). The endometrium is a pluripotent tissue, which shares a common embryological origin from the müllerian ducts with subjacent myometrium. It is composed of glands and stroma, with cytokeratin filament-containing epithelial tissue and vimentin-containing mesenchymal tissue, as in adenomyosis (Moll et al., 1983). Progenitor cells deposited in the peritoneal cavity by retrograde menstruation can possibly lead to the focal uterine adenomyosis. Alternatively, adenomyosis can differentiate from multipotent stem cells originated from bone marrow and other sources.

More recently, it has been demonstrated that adult stem cells are activated by tissue injury, promoting ectopic endometrial implants through endometrial stem/progenitor cell niche disruption (Gargett, 2007). However, more research is required to establish a role for endometrial stem/progenitor cells in the initiation and progression of adenomyosis (Gargett et al., 2016).

Pathogenic mediators

The main mechanisms involved in adenomyosis include sex steroid hormone aberrations, proliferation and fibrosis, inflammation and neuroangiogenesis (**Figure 1**), which in part explain the clinical symptoms of pain, AUB and infertility.

Sex steroid hormone aberrations

Steroid hormones are involved in the pathogenesis of adenomyosis. The uterine dysfunction may be the result of local hyperestrogenism with normal peripheral oestradiol levels (Urabe et al., 1989). This hormonal status represents the 'primum movens' of a chain of key events. Indeed, polymorphisms of the ER-α gene causing increased receptor activity are associated with a risk of adenomyosis (Oehler et al., 2004). The invagination process and overall 'spreading' of adenomyosis into the myometrium are suggested to be promoted by the non-cyclic and anti-apoptotic activity of the basalis, which is associated with increased oestrogen receptor (ER) and Bcl-2 gene expression in the adenomyosis foci throughout the menstrual cycle (Kitawaki, 2006). Local hyperestrogenism leads to increased peristalsis of the subendometrial myometrium, imposing supraphysiological mechanical strain on the cells near the fundo-cornual raphe. This state activates the TIAR system focally with further local production of oestradiol.

Sustained hyperperistaltic activity and chronic injury, proliferation and inflammation delay the healing process and result in increased numbers of foci. Hence, local areas of the basal endometrium, because of accumulation or expansion of such sites, start functioning as an endocrine gland that produces oestradiol. The hyperestrogenism is suggested to result from the activation of aromatase and sulphatase. This finding is reflected by the increased levels of oestradiol in menstrual blood, but not in peripheral blood of women with adenomyosis (Rižner, 2016). A vicious cycle is generated by a paracrine effect resulting from focal oestrogen production, presumably mediated by endometrial oxytocin and its receptor, which increases uterine peristalsis. Furthermore, the altered regulation of 17ß-hydroxysteroid dehydrogenase type 2 (17β-HSD2) in the eutopic endometrium of women with adenomyosis causes a decreased local oestrogen metabolism (Kitawaki et al., 2000) (**Figure 2**). The evidence that adenomyosis is an oestrogen-related process is also supported by the observation that postmenopausal women with breast cancer treated with tamoxifen have a higher rate of adenomyosis than those untreated (Cohen et al., 1998). Therefore, prolonged tamoxifen therapy, as a result of its oestrogenic effects, may promote the development of adenomyosis or its persistency in postmenopause (McCluggage et al., 2000).

Moreover, the increased expression of ER induces a down-regulation of progesterone receptors, a loss of their action and finally progesterone resistance (Jichan et al., 2010; Kitawaki et al., 2000). Ectopic and eutopic endometrium from women with adenomyosis show a reduction in progesterone receptor isoform B (PR-B) and IκB-α immunoreactivity, and an increase in nuclear p65, p50 and p52 expression. In addition, nuclear p65 immunoreactivity is correlated with heavy menstrual bleeding, while the severity of dysmenorrhoea is significantly associated with both decreased PR-B and increased nuclear p65 immunoreactivity in ectopic endometrium of women with adenomyosis (Nie et al., 2009) (**Figure 2**).

The immunoreactivity to deoxyribonucleic acid methyltransferases (DNMTs) in adenomyosis differs significantly from that in normal endometrium, suggesting that adenomyosis may be a disease caused

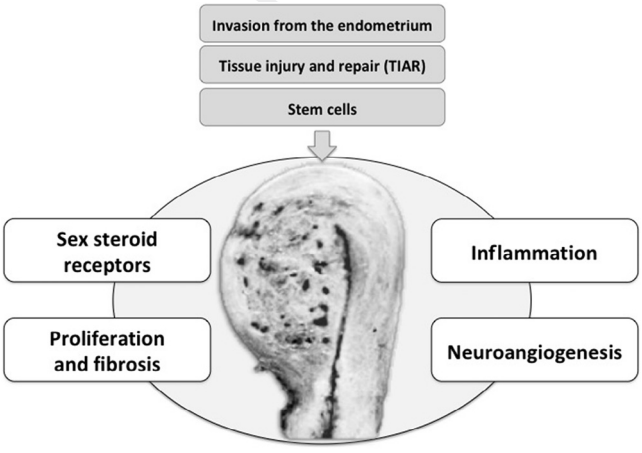

Figure 1 – Pathogenetic mechanisms of adenomyosis.

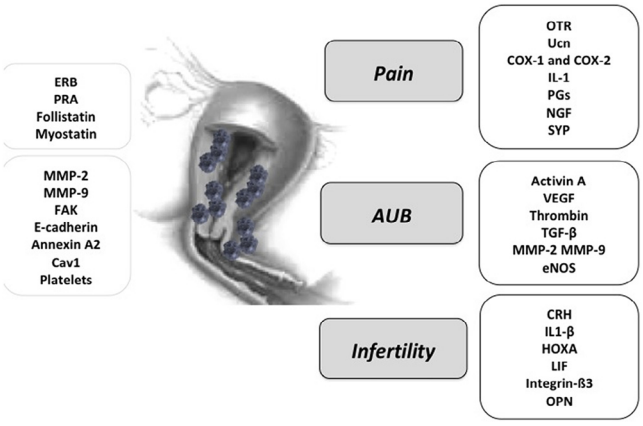

Figure 2 – Pathogenic mediators in adenomyosis.

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by epigenetic dysregulation of genes. DNMT1 and DNMT3B expression has been shown to be higher in ectopic endometrium, while DNMT3A levels are reduced in both eutopic and ectopic endometrium. DNMT1 levels in eutopic endometrium are positively associated with heavier menses. The correlation between DNMT3B and the severity of dysmenorrhoea suggests a role for DNMTs in adenomyosis-induced dysmenorrhoea (Liu and Guo, 2012). Furthermore, the expression profile of long non-coding RNA (lncRNA) – a key regulator of gene expression – is altered in eutopic endometrium of women with adenomyosis (Jiang et al., 2016a). In addition, studies on expression and localization of class I histone deacetylases (HDACs) have demonstrated that, compared with the normal endometrium, HDAC1 and HDAC3 expression was higher in both eutopic and ectopic endometria of women with adenomyosis. Similarly, HDAC2 expression in the eutopic endometrium was found to be associated with the severity of dysmenorrhoea. These findings suggest the potential involvement of HDACs in the pathogenesis of adenomyosis (Liu and Guo, 2012; Liu et al., 2012). Consistent with these observations, the promoter of PR-B has been reported to be methylated, rendering its silencing, which may be responsible for the well-known progestin resistance in adenomyosis (Jichan et al., 2010). Encouragingly, the use of valproic acid, a histone deacetylase inhibitor, has been reported to be efficacious in treating refractory adenomyosis, alleviating dysmenorrhoea and reducing the uterus size (Liu and Guo, 2008; Liu et al., 2010). Despite these promising results and the extensive *in vitro* and *in vivo* data favouring the use of HDAC inhibitors (Jichan et al., 2010; Liu and Guo, 2011), so far there has been no independent validation and, more dishearteningly, no one has expressed interest in conducting clinical trials to evaluate the efficacy of valproic acid. Given that the patent for valproic acid expired a long time ago, the lack of interest may simply reflect the lack of any financial incentive to pursue this drug.

Proliferation and fibrosis

An increased expression of growth factors (transforming growth factor β family, TGF-β) may play a role in the development of adenomyosis. Myostatin, follistatin and activin A expression are increased in adenomyotic nodules and may affect proliferation of endometrial glands/stroma and of surrounding myometrial cells (Carrarelli et al., 2015). Myocytes are the main target of myostatin and their proliferative activity is modulated by these growth factors. Adenomyosis is characterized by hyperplasia of myometrial cells surrounding endometrial stroma and glands that may be linked to the myostatin/follistatin overexpression.

Activin-related proteins are also key regulators of tissue remodelling and repair. Up-regulation of these molecules in adenomyotic tissue may be related to myometrial response to ectopic endometrial cell invasion. There is strong evidence that myostatin, activin A and TGF-β normally inhibit muscle growth and promote muscle protein loss in disease states, acting as powerful catabolic stimuli. Binding of these TGF-β ligands to muscle cell surface receptors leads to muscle proteolysis (Zhou et al., 2012), which can further support the invagination theory in a 'permissive' myometrium. Such myometrium is able to produce soluble factors (cytokines, chemokines, or other soluble molecules) that enhance the migration of stromal cells.

Furthermore, the mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPKs/ERKs) and phosphoinositide 3-kinase/mammalian target of rapamycin/AKT (PI3K/mTOR/AKT) cell-signalling pathways appear to be involved in proliferation of uterine smooth muscle cells (uSMCs) of women with adenomyosis (Streuli et al., 2015) (**Figure 2**).

According to the invagination theory, adenomyosis results from the increased invasiveness of the endometrial cells. It has been reported that oestrogen-induced epithelial-to-mesenchymal transition (EMT), a developmental programme exploited by cancer cells for their invasive and metastatic capacity, is crucial to the pathogenesis of adenomyosis (Chen et al., 2010b; Khan et al., 2014; Oh et al., 2013). EMT is characterized by loss of E-cadherin and apical–basal cell polarity, increased expression of mesenchymal markers, including fibronectin, N-cadherin and vimentin. Hence, cells acquire migration and invasion abilities (Polyak and Weinberg, 2009). Recent studies have shown that focal adhesion kinase (FAK) is involved in EMT regulation (Serrels et al., 2011). The expression of FAK was shown to be higher in the eutopic endometrium of women with adenomyosis compared with controls and FAK levels are positively correlated with dysmenorrhoea and pelvic pain (Mu et al., 2015a). In adenomyosis the activation of FAK potentially promotes EMT and invasion and metastasis of endometrial cells by dysregulation of E-cadherin. It is possible that FAK is vital in transforming the eutopic endometrium of adenomyosis to be more resilient to surviving, adhering and growing in the ectopic sites (Mu et al., 2015a).

Among a group of dysregulated oestrogen-responsive proteins identified in adenomyosis, annexin A2 (ANXA2) was shown to be significantly up-regulated in the ectopic rather than in the eutopic endometrium. Overexpression of ANXA2 is strongly correlated with markers of EMT and dysmenorrhoea severity in patients with adenomyosis. In an *in vitro* adenomyosis model, functional analysis indicates that oestrogen could considerably up-regulate ANXA2 and induce EMT. Increased expression of ANXA2 promotes phenotypic mesenchymal-like cellular changes, with structural and functional alterations mediated by β-catenin/T-cell factor (Tcf) signalling and angiogenesis enhancement through the HIF-1α/VEGF-A pathway (Zhou et al., 2012).

Similarly, loss of stromal caveolin (CAV1), a factor related to tumour progression, is involved in the pathogenesis of adenomyosis and adenomyosis-related dysmenorrhoea. Immunochemical examination of stromal CAV1 showed a significantly lower expression in the ectopic endometrium of patients with adenomyosis than that of paired eutopic endometrium or normal controls. CAV1-depleted primary endometrial stromal cells (ESCs) and endometrial epithelial cells (EECs) demonstrated a significantly elevated proliferation rate, enhanced migration and invasive capacity. Indeed, in women with more severe dysmenorrhoea a significantly lower stromal CAV1 expression in the eutopic endometrium was observed, suggesting a negative correlation with severity of pain symptoms in patients with adenomyosis. Conversely, a positive correlation between stromal RANTES expression in the eutopic endometrium and severity of menstrual pain was reported in patients with adenomyosis (Zhao et al., 2013).

Platelets

One salient hallmark of adenomyotic lesions is cyclic bleeding, as in endometriotic lesions (Brosens, 1997). Yet bleeding is a cardinal feature of tissue injury. Once there is a tissue injury, tissue repair, an evolutionarily conserved mechanism in all organisms, will ensue. As such, platelets are involved in mammals when experiencing tissue injury. It has been shown recently that, indeed, platelets are highly aggregated in endometriosis and seem to play important roles in the development of endometriosis (Ding et al., 2015). In fact, activated platelets drive the EMT, fibroblast-to-myofibroblast transdifferentiation

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(FMT), smooth muscle metaplasia (SMM) and fibrogenesis in endometriosis through the activation of the TGF-β1/Smad3 signalling pathway (Zhang et al., 2016a). Serial observational studies of both mouse and baboon models of endometriosis lend strong support to this idea (Zhang et al., 2016b, 2016c). In humans, evaluation of the ovarian endometrioma cyst fluid also provides data that are consistent with this notion (Guo et al., 2015a). Also consistent with this notion, anti-platelet therapy has been shown to be effective in mice with induced endometriosis (Guo et al., 2015b). In a serial experimentation of mouse adenomyosis, progressive EMT, FMT, SMM and fibrogenesis have been found (Shen et al., 2016). In human adenomyosis, it has been found that indeed platelets are aggregated in adenomyotic lesions and the data are consistent with gradual but progressive EMT, FMT, SMM and fibrosis (Liu et al., 2016). Similar to endometriosis, anti-platelet therapy has also been found to be efficacious in treating mice with induced adenomyosis (Zhu et al., 2016). Because of FMT and SMM, the enlarged uterus that is characteristic of adenomyosis may well be the result of platelet-driven cellular transdifferentiation. This heterogeneity and enlargement of smooth muscle cell populations in the myometrium resulting from adenomyosis may lead to increased uterine contractility on the one hand and the lack of synchronized contraction on the other, eliciting pain perception.

Other data also give credence to this view. Valproic acid, which has been shown to be promising in treating human adenomyosis (Liu and Guo, 2008; Liu et al., 2010), turns out to be anti-platelet (Davidson et al., 2011), as are resveratrol (Yang et al., 2008) and andrographolide (Lien et al., 2013; Lu et al., 2011), which have been shown to be promising in treating adenomyosis in animals (Zhu et al., 2015) and humans (Liu et al., 2015).

Pelvic pain

Uterine contractility and oxytocin receptors

Women with symptomatic adenomyosis exhibit increased uterine contractility associated with dysmenorrhoea (Mao et al., 2011). In uSMCs the contractile amplitude and oxytocin receptor (OTR) expression levels were significantly higher in women with adenomyosis than in those without, with a correlation with intensity of dysmenorrhoea (Guo et al., 2013; Nie et al., 2010). Conceivably, a hyperactive uterus, as manifested with dysperistalsis or even spasm during menstruation, coupled with increased innervations, should suffice to cause dysmenorrhoea.

Immunohistochemical examination of both OTR and vasopressin receptor (VP1αR) expression in the endometrium, myometrium and adenomyotic lesions demonstrated morphological changes and overexpression of OTR in the adenomyosis-surrounding myometrium, while VP1αR was expressed in myometrial cells and blood vessels. Thus, morphological changes and increased myometrial OTR expression suggest that dysperistalsis plays an essential role in the development of adenomyosis and dysmenorrhoea (Mechsner et al., 2010).

The changes in the expression or activity of potassium channels in uSMCs can cause inadequate membrane depolarization, resulting in abnormal uterine activity (Brainard et al., 2007). In uSMCs from adenomyotic tissues, the expression of large conductance calcium- and voltage-sensitive potassium channel (BKCa)-α/β subunits and voltage-gated potassium channel (Kv) 4.2 and Kv4.3 was significantly higher than those in the control group. The abnormal uterine smooth muscle contractility may cause a change in the microcirculation of the uterus, accumulating inflammatory factors. Those mediators contribute to impairing the endometrium further, worsening the pain symptoms (Shi et al., 2016).

Among signalling pathways involved in smooth muscle contraction, RhoA and ROCK-I mRNA and protein expression have a typical menstrual cycle-dependent pattern in the normal JZ. Conversely, in adenomyosis, the levels of RhoA and ROCK-I are increased, without the typical cyclic change. RhoA/ROCK-I signalling may be enhanced by oestrogens, affecting uterine JZ contraction in adenomyosis (Wang et al., 2016).

Inflammatory peptides and prostaglandins

A clear involvement of inflammation in the pathogenesis of adenomyosis was suggested by the observation of high expression of IL-1β, CRH and UCN in adenomyotic nodules (Carrarelli et al., 2016). The increased expression of CRH and UCN may be part of the local responses to the invading endometrial cells. Mast cells activated by CRH and UCN may play a role in the development of inflammation in adenomyosis. Because CRH/UCN has been shown to activate COX-2 in other tissues, the high expression of CRH and UCN in adenomyosis may also lead to increased prostaglandin synthesis (Carrarelli et al., 2016).

The hypothesis that NF-κB plays a critical role in the pathogenesis of adenomyosis is supported by the evidence of increased expression of the NF-κB p65 subunit in the eutopic endometrium and adenomyotic nodules (Li et al., 2013). In addition, stromal cells of the eutopic endometrium and adenomyotic tissues showed an increased immunoreactivity of both nuclear and the cytoplasmic NF-κB p65 subunit, while in the glandular cells only the nuclear staining of the NF-κB p65 subunit was found to be higher than in controls (Park et al., 2016).

The mechanism of tissue traumatization and healing physiologically involves local production of interleukin-1 (IL-1), which induces the cyclooxygenase-2 enzyme (COX-2), causing the production of prostaglandin E_2 (PGE_2). Hence, the steroidogenic acute regulatory protein (STAR) and the P450 aromatase are activated. Thus, the aromatization of testosterone into oestradiol contributes to a local hyperestrogenic state with its proliferative and healing effects via the oestrogen receptor (ERβ) (Leyendecker et al., 2009).

Hyperestrogenism was also shown to stimulate the production of IL-10, a cytokine with immunosuppressive abilities. High expression of IL-10 was demonstrated in both the eutopic and ectopic endometrium of women with adenomyosis. This observation may explain the persistence of the ectopic foci within the myometrium without elimination by the immune system of the host (Wang et al., 2009).

The TLR4 signalling pathway in stromal cells of the eutopic and ectopic endometrium is postulated to be essential for the pathogenesis of adenomyosis. TLR4 signalling, activated by endogenous ligands, promotes the secretion of various cytokines and growth factors, stimulates endometrial cell proliferation as well as recruiting and activating immune cells (macrophages, DCs, NK cells). Further induction of stromal cell proliferation and invasion amplifies local inflammatory response, and eventually leads to the development of adenomyosis (Guo et al., 2016) (**Figure 2**).

Neurogenic factors

The myometrium is innervated by a subserosal plexus and a plexus at the endometrial–myometrial junction (Krantz, 1959). The functional layer of the endometrium is mainly innervated by sensory unmyelinated C nerve fibres that may be activated or sensitized by inflammatory mediators released from the endometrium, resulting

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in neurogenic inflammation. PE_2, prostacycline and norepinephrine released from adrenergic fibre endings can sensitize sensory C (Apfel, 2000).

Zhang et al. (2009, 2010) reported the presence of PGP9.5-positive nerve fibres in the functional layer of the endometrium of women with adenomyosis or uterine fibroids and associated pain symptoms, while they were absent in those with asymptomatic adenomyosis or uterine fibroids. Furthermore, neurofilament protein (NF)-positive cells were detected in the endometrium and myometrium of women with myoma and adenomyosis, playing a potential role in pain generation (Choi et al., 2015). In addition, a positive correlation between severity of pain and PGP9.5 and NF staining in the myometrium was observed in women with painful adenomyosis (Lertvikool et al., 2014).

Nerve growth factor (NGF) is involved in pain generation, neural plasticity, immune cell aggregation and release of inflammatory factors. In a mouse model of adenomyosis, NGF-β and its receptors expressed in the uterus and in dorsal root ganglia were found to be higher in the older mice-developed adenomyosis model group than in controls. The gradual increase of NGF-β and its receptor levels as the disease worsens suggests the role played by NGF-β in adenomyosis pathogenesis (Li et al., 2011). High expression of NGF, synaptophisin (SYN) and MAP2 mRNAs in adenomyotic nodules implicated possible neurogenesis in adenomyosis and its associated pain. Protein expression of CRH, NGF and SYN in adenomyotic nodules was also confirmed by immunohistochemical and immunofluorescence analyses. Furthermore, urocortin-induced NGF mRNA expression in cultured human ESCs confirms a link between inflammatory and neurogenic pathways (Carrarelli et al., 2016).

Mice with induced adenomyosis also show a decreased number of glutamate decarboxylase (GAD)65-expressing neurons with resulting loss of GABAergic inhibition and hyperalgesia. The number of these neurons is increased by treatment with epigallocatechin-3-gallate (EGCG), which also reduces the expression of inflammatory mediators and OTR in the ectopic endometrium or myometrium, improving hyperalgesia. In addition, EGCG treatment reduces the number of macrophages infiltrating into the ectopic endometrium, while it increases the expression of PR-B (Chen et al., 2013). Thus, adenomyosis-induced pain resembles neuropathic pain with remarkable central plasticity (Chen et al., 2014).

The glycoprotein neural cell adhesion molecule (NCAM), also known as CD56, is highly expressed in endometrial glandular epithelium in patients with adenomyosis, with a positive correlation between epithelial staining intensity and dysmenorrhoea. The increased secretion of CD56 stimulates nerve growth in the stroma, suggesting its role in the development of menstrual pain in adenomyosis (Wang et al., 2015). The severity of dysmenorrhoea also correlates with the Slit–Robo system, known for its role in neuronal development. Slit and Robo immunoreactivity were found to be increased in the ectopic endometrium of women with adenomyosis and this system, with microvessel density (MVD) in the eutopic endometrium, has been reported to be a significant predictor for dysmenorrhoea severity (Nie et al., 2011).

In adenomyotic lesions and eutopic endometrium the expression of Cyr61, a secreted ECM-associated signalling protein of the CCN family, is correlated with age, number of spontaneous labours, PBAC score, VAS score, uterine volume, adenomyosis type, and concurrent endometriosis. Thus, Cyr61 may be indirectly related to the degree of dysmenorrhoea and involved in the pathogenesis of adenomyosis (Zhang et al., 2016d).

Because the ectopic endometrium is reported to secrete potent platelet activators, such as thrombin and thromboxane A2 (TXA2) (Guo et al., 2016), so do activated platelets, which are known to be activated in endometriosis (Ding et al., 2015) and adenomyosis (Shen *et al.*, 2106). TXA2 was recently reported to be a neurotrophic factor and may be responsible for increased innervations in endometriosis (Yan et al., 2016) (**Figure 2**).

Abnormal uterine bleeding (AUB)

Recent evidence has shown that activin A modulated endometrial vascularization by stimulating ESCs to produce vascular endothelial growth factor (VEGF), one of the most potent angiogenic factors (Rocha et al., 2012). Both activin and follistatin were suggested to be involved in the development of dysmenorrhoea and heavy menstrual bleeding. In adenomyotic nodules, activin A, myostatin, follistatin and both activin type II receptors are highly expressed. Compared with control endometrium, the expression of follistatin and type II receptors is elevated in eutopic endometrium from patients with adenomyosis (Carrarelli et al., 2015). The increased activin A expression observed in adenomyotic nodules potentially alter the microenvironment in adenomyosis by affecting the inflammatory response and neoangiogenesis. An autocrine/paracrine effect of these growth factors in adenomyosis is also indicated by the increased local ActRIIa and ActRIIb expression (Carrarelli et al., 2015).

In both eutopic and ectopic endometria of adenomyosis, the expression of MMP-2, MMP-9 and VEGF was significantly greater than that in normal endometrium with a positive correlation between VEGF and metalloproteinase expression. This observation suggests a role played by MMP-2, MMP-9 and VEGF in the invasion of endometrial tissues into the myometrium and in angiogenesis in adenomyotic implants. The serum levels of VEGF in patients with adenomyosis were proposed to predict the prognosis of adenomyosis after interventional therapies (Mu et al., 2015b).

Another factor involved in angiogenesis in adenomyosis is the retinoid-interferon (IFN)-induced mortality 19 (GRIM-19). A recent study showed that GRIM-19 was down-regulated in the eutopic endometrium and the levels were further reduced in the endometrial glandular epithelial cells of adenomyotic lesions. Down-regulation of GRIM-19 promotes angiogenesis through the activation of both pSTAT3 (Y705) and its dependent gene VEGF (Wang et al., 2016), causing a higher microvessel density in eutopic and ectopic endometria (Goteri et al., 2009).

Endothelial nitric oxide synthase (eNOS) plays an essential role in the occurrence of bleeding. The expression of eNOS in endometrium and myometrium specimens is higher in the uterus with adenomyosis than that in controls. Furthermore, the expression of eNOS is significantly higher in patients with dysmenorrhoea and menorrhagia (Oh et al., 2013).

Tissue factor (TF) is involved in heavy menstrual bleeding and dysmenorrhoea in adenomyosis and an increased expression has been shown in both eutopic and ectopic endometria, with a positive correlation with heavy menses and severity of dysmenorrhoea (Liu and Guo, 2011; Liu et al., 2011). This is consistent with the recent finding that platelets are aggregated in adenomyotic lesions (Liu et al., 2016) (**Figure 2**).

Infertility

Adenomyosis is described as a cause of infertility and negative assisted reproductive technologies outcomes (Vercellini et al., 2014), possibly due to the alteration of the normal myometrial architecture that disturbs the uterine environment, uterine peristalsis and sperm transport (Leyendecker et al., 1996). These abnormalities even-

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tually result in implantation failure (Campo et al., 2012a; Sunkara and Khan, 2012).

Accumulating evidence suggests that adenomyosis is strongly associated with subfertility in reproductive age women. Dysregulation of a number of implantation-associated factors, such as HOXA10, LIF, MMP2, IL-6, cytochrome P450 and RCAS1, in the eutopic endometrium in women with adenomyosis leads to reduced endometrial receptivity and impaired decidualization (Campo et al., 2012b; Fischer et al., 2011; Xishi et al., 2010; Zhou et al., 2012).

HOXA10 gene expression is decreased in the secretory phase endometrium of women with adenomyosis, suggesting a potential mechanism for impaired implantation observed in these women (Fischer et al., 2011). Similarly, a dysregulation of leukaemia inhibitory factor (LIF) in the endometrium and uterine flushing fluid of women with adenomyosis has been observed during the implantation window (Xiao et al., 2013). Orphan nuclear receptor NR4A is a novel regulator of decidualization, acting as ligand-independent transcription factor and activating target genes in human ESCs. In adenomyotic tissues, down-regulation of NR4A receptor and FOXO1A were found to impair decidualization (Jiang et al., 2016b).

Inflammation is another key factor mediating adenomyosis-associated infertility. An increased expression of IL-1β and CRH in the eutopic endometrium of patients with adenomyosis suggests an involvement of endometrial inflammatory pathways in infertility (Carrarelli et al., 2016). Indeed, the cellular and humoral immunity in a eutopic endometrial microenvironment in adenomyosis and in the endometrium of unaffected women has been shown to be different (Benagiano et al., 2014). Increased free radical metabolism with release of reactive oxygen species by macrophages and altered expression of endometrial pro-oxidant and anti-oxidant enzymes are signals of increased inflammatory responses in the endometrium of adenomyosis (Ishikawa et al., 1993; Ota et al., 1998; Van Langendonckt et al., 2002).

The eutopic endometrium displays a dysregulation of immune factors, markers of apoptosis or proliferation, inflammatory mediators, and oxidative stress with low uterine receptivity (Campo et al., 2012a). Members of the integrin family of cell adhesion receptors play a vital role in cell–cell interactions at the conceptus–endometrial interface, involving the participation of extracellular matrix. Integrin β3 has been shown to be elevated in the human endometrium at implantation and, together with osteopontin (OPN), its main ligand, has been proposed as a biomarker of uterine receptivity. In the endometrium of adenomyotic patients, integrin β3 and OPN levels were significantly lower than those in control endometrium (Xiao et al., 2013).

In adenomyosis, a number of cellular and humoral immune responses are observed, causing an immunological 'vicious cycle' in the endometrium. The activation of the immune system includes the expression of cell surface antigens or adhesion molecules, an increased number of macrophages, and deposition of immunoglobulins and complement components. In addition, endometrial cells expose heat shock proteins, showing signs of immunological stress and autoantibodies are found very frequently in the peripheral blood of women with adenomyosis (Ota et al., 1998). These abnormal immune responses are proposed to be involved in poor reproductive performance in adenomyosis (Ota and Igarashi, 1993) (**Figure 2**).

Conclusions

The pathogenic mechanisms of adenomyosis development are still unclear and, because there are different phenotypical expressions of adenomyosis, it is not proven yet that all the evidence available may be applied to different forms of the disease. However, sex steroid hormones, inflammation, neonagiogenesis, growth factors, ECM enzymes and neurogenic factors are key pathogenic mediators of pain, AUB and infertility. More research is needed to better understand the pathophysiology and the early pathways implicated in the initiation of adenomyosis, in order to develop adequate therapeutic strategies.

Uncited references

Barrier et al, 2004, Choi et al, 2010, Garcia-Segura, 2008, Laoag-Fernandez et al, 2003, Li et al, 2006, Tokushige et al, 2006

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