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Endocrinology of Uterine Fibroids: Steroid Hormones, Stem Cells, and Genetic Contribution

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Abstract

Purpose of Review—Uterine fibroids are extremely common, and can cause significant morbidity, yet the exact etiology of these tumors remains elusive and there are currently no long-term treatments available. In this review we aim to provide an overview of steroid hormones, genetic abnormalities, and stem cells in the pathogenesis of uterine fibroids.

Recent Findings—A universal feature of fibroids is responsiveness to estrogen and progesterone, and most of the currently available therapies exploit this characteristic. Ulipristal acetate has recently shown particular promise for providing long-term relief from uterine fibroids. Additionally, fibroid stem cells were isolated and appear to be necessary for growth. The recent discovery of somatic mutations involving MED12 or HMGA2 in the majority of fibroids and the links to their pathophysiology were also significant advances.

Summary—The recent shift in focus from hormones to fibroid stem cells and genetic aberrations should lead not only to a deeper understanding of the specific etiology of fibroids, but also to the discovery of new therapeutic targets. Targeting the products of genetic mutations or fibroid stem cells has the potential to achieve both better control of current tumors and the prevention of new fibroids.

Keywords

Fibroids; steroid hormones; stem cells; genetics

Introduction

Uterine fibroids occur in up to 80% of reproductive-age women, causing significant morbidity in up to 30% of women[1-4]. In the United States, more than 200,000 surgical procedures are performed for the treatment of fibroids, with yearly cost estimates of $5.9-34.4 billion[5]. Despite this impressive prevalence, the exact etiology of uterine fibroids remains elusive and there are currently no long-term treatments available. Studies have suggested that fibroids are monoclonal tumors developed from a single myocyte[6, 7], but the inciting event for neoplastic transformation of a myocyte is currently unknown.

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Tumor growth is characterized by slow proliferation with concurrent deposition of abundant extracellular matrix (figure 1), usually in a steroid-hormone dependent manner[8, 9]. This review provides an overview of the current state of knowledge on the role of steroid hormones in fibroid development, treatments targeting steroid hormone action, and the more recent discoveries regarding genetic abnormalities and stem cells in the pathogenesis of uterine fibroids.

Steroid Hormones

A universal feature of fibroids is responsiveness to estrogen and progesterone, and most of the currently available therapies exploit this characteristic.

Estrogen and Aromatase

Estrogen upregulates gene expression of multiple growth factors, collagens, and the estrogen and progesterone receptors (ER, PR), all thought to play a role in fibroid pathogenesis[10-12]. Estrogen action is mediated through its nuclear receptors, ERα and ERβ, expressed in both myometrial and fibroid tissue[13-15]. ERα is a more potent activator of transcription and is thought to be regulated by ERβ, although much remains unknown about the exact roles of the two receptors and their interactions[16]. Additionally, there have now been several studies that have reported specific ERα polymorphisms that increase susceptibility to uterine fibroids[17, 18].

Fibroids respond to estrogen in the bloodstream as a result of ovarian steroidogenesis, and also produce estrogen *in situ* through local conversion of androgens by aromatase[19]. Fibroids have been shown to have higher estrogen levels then adjacent myometrium, and correspondingly increased aromatase and 17β-HSD type 1 levels[19-22]. Interestingly, aromatase RNA is not found in the myometrium of women without fibroids[19]. The addition of androstenedione alone to cultured fibroid cells leads to estradiol production, with resultant cellular proliferation comparable to that caused by the addition of estradiol alone, suggesting that fibroids are capable of producing sufficient estrogen to sustain their own growth[21]. The addition of aromatase inhibitors to fibroid cell culture reverses this effect[21].

Progesterone

In addition to estrogen and aromatase, there is accumulating evidence that progesterone plays a critical role in uterine fibroid expansion[23] and is essential for estrogen-related fibroid growth[24-28]. Progesterone acts through two isoforms of PR, PR-A and PR-B, both of which exhibit higher expression in fibroids compared with adjacent myometrium[29-31]. Similar to ER, relatively little is known about the specific roles and interplay of PR-A and PR-B in fibroids.

In support of a key role for progesterone, markers of proliferation and mitotic counts are highest in fibroid tissue during the luteal phase[25, 28] and fibroid proliferative activity in postmenopausal women has been shown to increase significantly with combined estrogen and progestin replacement but not with estrogen replacement alone[25]. In a xenograft mouse model, Ishikawa et al. showed that estrogen regulates expression of PR via ERα, and

progesterone directly stimulates fibroid growth[26]. In this model, estrogen with progesterone stimulated both fibroid cell proliferation and extracellular matrix formation, and these effects were abolished by co-treatment with a progesterone receptor antagonist[26]. These findings suggest a more permissive role for estrogen, allowing fibroid responsiveness to progesterone via induction of PR[4, 26].

Recently, in a xenograft model, Qiang, et al. (2014) demonstrated that treatment with estrogen and progesterone resulted in the formation of extracellular matrix production via downregulation of miR-29b[32*]. Gene expression of miR-29b has been consistently shown to be lower in fibroid tissues compared with adjacent normal myometrium tissues, both *in vitro* and *in vivo*[32*-34] and increasing mir-29b levels in fibroid cells with mir-29b lentivirus decreased levels of collagen 1a1[32*]. Lastly, estrogen with progesterone, but not estrogen alone, decreased miR-29b expression, suggesting a role for progesterone in promoting uterine fibroid growth via miR29n downregulation[32*].

Medical Treatments

While the mainstay of fibroid treatment has traditionally been surgical, much recent research has focused on less invasive medical therapies. Historically, GnRH agonists were first-line therapy for fibroids, but they can cause severe menopausal symptoms, and cannot be used long-term. A number of reviews are available on non-surgical management of fibroids[35,36*,37-39], so the topic will not be reviewed in depth here. Currently available therapies are summarized in table 1. As proof of principle of the above-mentioned hormonal aspects, we will briefly review aromatase inhibitors and selective progesterone receptor modulators (SPRMs), highlighting the exiting recent progress with ulipristal acetate.

Aromatase Inhibitors

Because aromatase is thought to play such a critical role in estrogen production in fibroids, aromatase inhibitors are a logical treatment choice. Non-steroidal aromatase inhibitors reversibly bind the aromatase enzyme, decreasing binding by androstenedione or testosterone and thus decreasing conversion to estradiol[40, 41]. While the original aromatase inhibitors were relatively nonselective and fraught with side effects, third generation aromatase inhibitors are more selective and have superior bioavailability and side effect profiles. Anastrozole and letrozole are able to inhibit >98% of aromatase activity[40, 42], and have been shown to result in significant reduction of fibroid volume and improvement in symptoms in multiple clinical trials[43-46]. Moreover, aromatase inhibitors avoid the side effects of the severe hypoestrogenism caused by GnRH agonists, particularly hot flushes[44].

While most women tolerate aromatase inhibitors relatively well, there are potential side effects. The most commonly reported side effects include hot flashes and musculoskeletal pain. Importantly, aromatase inhibitors are often used off-label in the follicular phase for ovulation induction or controlled ovarian stimulation, necessitating contraception in women not desiring conception[47]. Although there have been conflicting results regarding the potential for systemic hypoestrogenism with prolonged aromatase inhibitor use[44, 48], there is concern for both increased bone loss and cardiovascular risk with long-term

aromatase inhibitor use, particularly in younger patients[42]. The breast cancer literature has also brought some questions as to the utility of aromatase inhibitors, reporting both decreased effectiveness in overweight and obese women and the development of resistance over time[40, 42]. Additionally, the effects of aromatase inhibitors are only temporary, and fibroids regrow with cessation of treatment, albeit to smaller volumes[44]. Taken together, the current evidence suggests that aromatase inhibitors are, at best, a short-term solution in select populations of women.

Selective Progesterone Receptor Modulators

All of the SPRMs that have been studied in clinical trials—mifepristone (RU486), asoprisnil (J867), ulipristal acetate (CDB2914), and telapristone acetate (CDB4124)—have been shown to reduce fibroid size and improve quality of life[49-51]. *In vitro*, fibroid cells treated with ulipristal acetate, telapristone acetate, or asoprisnil exhibit decreased cell proliferation and increased apoptosis[52-56], Moreover, asoprisnil and ulipristal both decrease extracellular matrix formation[55, 57, 58]. These effects are not seen with treatment of myometrial cells, suggesting tissue-specificity of these drugs. Both asoprisnil and ulipristal acetate also have high affinity for PR[59, 60], suggesting that the genome-wide binding status of PR liganded with ulipristal or asoprisnil should be further investigated.

Most recent research has focused on ulipristal acetate. Although the Food and Drug Administration has not yet approved ulipristal acetate for indications beyond contraception in the US, it has been approved in both Canada and Europe for the treatment of fibroids. Clinical trials have shown that, while GnRH agonist causes greater overall reduction in fibroid volume, ulipristal acetate has longer-lasting effects after cessation of treatment[8, 50, 61, 62]. Additionally, ulipristal acetate results in a lower incidence of hot flashes, impact on bone density, and suppression of E_2 levels when compared to GnRH agonist[62, 63]. Moreover, Donnez et al. recently reported that repeated 3-month courses of ulipristal resulted in amenorrhea in almost 90% of women and was well tolerated[64*, 65**]. This exciting study suggests that ulipristal could be the first long-term treatment for uterine fibroids.

Because SPRMs block progesterone action in the endometrium, concern has been raised that they may result in endometrial thickening and premalignant or malignant transformation. There are now studies showing that treatment with SPRMs does not appear to result in increased mitosis or atypia; however, asymmetry of stromal and epithelial growth and cystic, dilated glands have been reported, and are now classified as progesterone receptor modulator-associated endometrial change (PAEC)[49, 66, 67]. Encouragingly, one study observed reversal of these changes and return to normal endometrial histology six months after ulipristal acetate discontinuation[67], and the study of repeated courses of ulipristal did not report any increase in PAEC or other histological changes[65**], but longer term studies are needed to definitively understand the risks and side effects of SPRMs.

Somatic Stem Cells

Somatic stem cells were first discovered in myometrial tissues, where they are capable of both self-renewal and the production of tissue-specific daughter cells under the influence of

estrogen and progesterone[68-70]. More recently, small populations of fibroid cells consistent with somatic stem cells have also been isolated[71, 72]. Despite the fact that fibroids contain lesser stem cells than the myometrium[73], there is evidence that the fibroid stem cell population is essential for steroid hormone-dependent fibroid growth[71, 72]. In a mouse xenograft model, injected cell suspensions containing fibroid stem cells mixed with myometrial cells grew into substantially bigger tumors and had higher proliferation indices under the influence of estrogen and progesterone than injected suspensions containing only differentiated fibroid cells with myometrial cells[72]. Perhaps most interestingly, fibroid stem cells have minimal to no ER and PR expression, yet respond to estrogen and progesterone stimulation with tumor expansion. Additionally, fibroid stem cells cannot induce proliferation or tumor growth without the presence of differentiated fibroid or myometrial cells. These observations have led us to hypothesize that fibroid stem cells rely on paracrine signaling from surrounding mature myometrial and fibroid cells to facilitate estrogen and progesterone action[72].

The wingless-type (WNT)/β-catenin pathway was recently proposed by Ono et al.[74*] as a possible mechanism for paracrine interaction between fibroid stem and differentiated cells. In that study, mature myometrial cells secreted WNT ligands in response to estrogen and progesterone treatment, resulting in nuclear translocation of β-catenin in proximal fibroid stem cells. Intranuclear β-catenin increased expression of genes involved in growth and proliferation. Additionally, inhibiting WNT binding or β-catenin in fibroid stem cells resulted in significantly decreased tumor growth—an effect not seen in mature fibroid cells[74*].

Much remains to be explored in fibroid stem cells. Originally, fibroid stem cells were isolated using the Hoechst dye exclusion technique for side populations (SP)[75, 76], however, the SP technique is expensive, exhibits significant sensitivity to minor staining variations, and is detrimental to cell survival, making further study of fibroid cells difficult[77]. As a solution to these pitfalls, we recently reported a novel way of isolating fibroid stem cells using cell surface markers CD34 and CD49b[78**]. Cell sorting using antibodies to these cell surface proteins revealed 3 distinct cell populations: CD34+/CD49b+, CD34+/CD49b-, and CD34-/CD49b- cells (figure 2). CD34+/CD49b+ cells were highly enriched with stem cells whereas the other two groups did not contain any stem cells. Moreover, genes specific to stem cells, such as KLF4, NANOG, OCT4 were overexpressed in the CD34+/CD49b+ cells further suggesting that these cells are indeed stem cells[78**]. Interestingly, CD34+/CD49b- cells had intermediate levels of these stem cell factors compared to CD34-/CD49b- cells. Additionally, ER-alpha and PR were significantly underexpressed in CD34+/CD49b+ cells, consistent with prior studies on SP, and CD34+/CD49b- cells again showed intermediate expression levels between CD34+/CD49b+ and CD34-/CD49b- cells[78**]. Taken together, these results led us to hypothesize that CD34+/CD49b+ cells are largely fibroid somatic stem cells, capable of asymmetric division allowing both self-renewal and the production of intermediary daughter cells, or CD34+/CD49b-cells, which ultimately develop into fully differentiated fibroid cells, or CD34-/CD49b- cells. An unbiased genome-wide investigation to better characterize the three populations on a molecular level is currently underway and will hopefully lead to new therapeutic targets.

Genetic Abnormalities

Recent research suggests that most fibroids fall into one of four categories of mutations: MED12 mutations, FH inactivation, COL4A6-COL4A5 deletions, or HMGA2 overexpression[79, 80*]. In one study of HMGA2 and MED12 mutations in fibroids, the two mutations appear to be mutually exclusive, raising the possibility that different genetic abnormalities in fibroids actually represent separate pathophysiology[81]. In support of this hypothesis, HMGA2 aberrations are highly correlated with big fibroid tumors, whereas tumors with MED12 mutations tend to be smaller[82, 83]. Because of their possible role in stem cell action, we will focus on HMGA2 and MED12 mutations in this review.

HMGA2

Mutations involving HMGA2 are found in approximately 7.5% of fibroid tumors and HMGA2 overexpression is due to rearrangements involving chromosome 12q14-15[83]. In mouse neural stem cells, HMGA2 expression inhibits senescence by downregulating $p16^{INK4a}$, a suppressor of stem cell self-renewal[84]. Similarly, HMGA2 has been shown to downregulate $p14^{Arf}$, also a negative regulator of self-renewal, in fibroid cells[85]. Finally, uterine fibroids exhibit underexpression of Let-7, which is known to suppress HMGA2[86]. These findings have led us to hypothesize that the Let7-HMGA2-$p14^{Arf}$ pathway may play a significant role in fibroid stem cells when altered, resulting in increased self-renewal and decreased senescence.

MED12

In the largest study of MED12 mutations in fibroids, specific MED12 mutations were found in 70% of fibroids, although smaller studies have reported a prevalence anywhere from 48% to 92%[80, 87]. It has been shown that stem cells from fibroid tissue, but not from myometrial tissue, carry MED12 mutations, supporting our hypothesis that a genetic hit may explain the transformation of a myometrial stem cell to a fibroid stem cell[72]. MED12 regulates Wnt signaling by binding to β-catenin, making it possible that absence of or defects in MED12 in fibroid stem cells could lead to unregulated Wnt/β-catenin pathway-stimulated tumor growth[4, 88]. Moreover, MED12 deficiency, possibly in somatic stem cells, releases negative regulation of TGFβ signaling, resulting in increased proliferation in cancer cells[89, 90]. Taken together, this evidence suggests that MED12 deficiency could lead to activation of the Wnt/β-catenin and TGFβ pathway, thereby supporting stem cell renewal, proliferation, and fibrosis in uterine fibroids[4, 90-92].

Conclusions

Historically, the vast majority of fibroid research has focused on the role of steroid hormones in fibroid pathogenesis. The result of this work has been the development of medical treatment options targeting steroid hormones, such as GnRH agonists, aromatase inhibitors and anti-progestins. To date, we have not found a medical treatment for uterine fibroids that results in permanent tumor shrinkage or eradication, or that can be used long-term with minimal side effects, although the data on ulipristal acetate look promising. Finding an effective, long-term treatment for fibroids could have great public health

implications, given their high prevalence and associated medical costs. The recent shift in focus from hormones to fibroid stem cells and genetic aberrations should lead not only to a deeper understanding of the specific etiology of fibroids, but also to the discovery of new therapeutic targets. Targeting the products of genetic mutations or fibroid stem cells has the potential to achieve both better control of current tumors and the prevention of the development of new fibroids.

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Key Points

- A universal feature of fibroids is responsiveness to estrogen and progesterone.
- Ulipristal could be the first long-term treatment for uterine fibroids.
- Fibroids contain somatic stem cells that are necessary for growth, but require paracrine signals from surrounding matures cells.
- Genetic mutations, particularly those affecting MED12 and HMGA2, likely explain some of fibroid pathogenesis.
- Targeting the products of genetic mutations or fibroid stem cells has the potential to achieve both better control of current tumors and the prevention of the development of new fibroids.

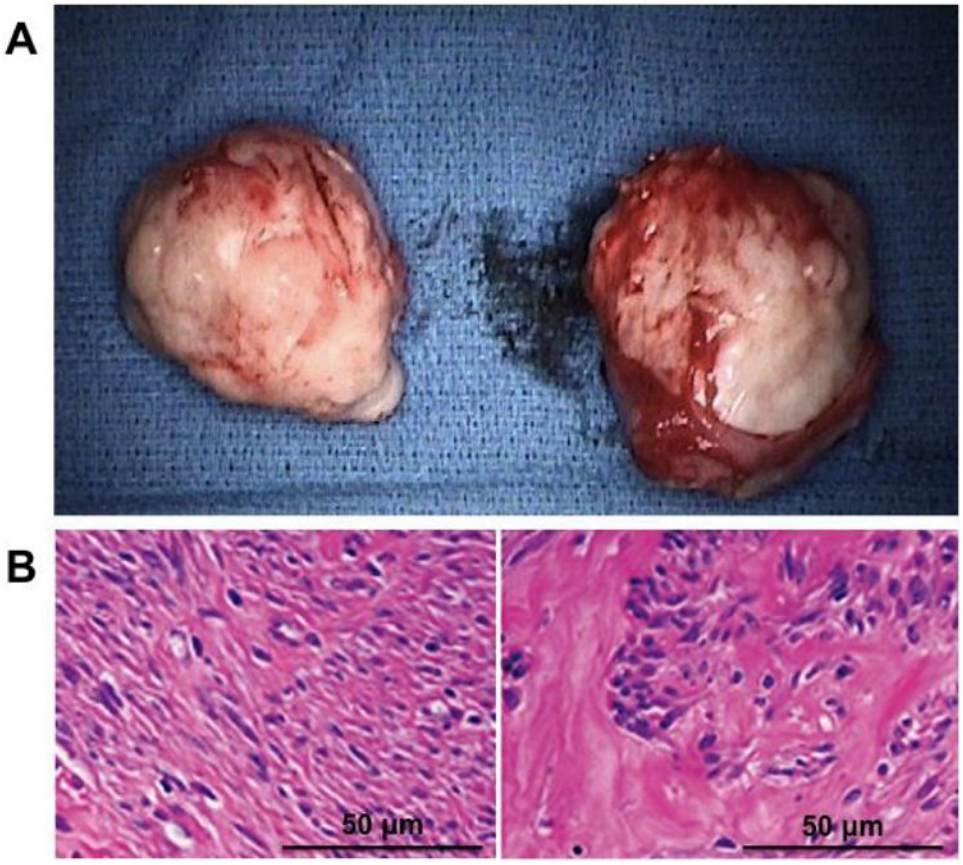

Figure 1.

(A) Gross fibroid specimen after surgical removal; **(B)** Representative hematoxylin and eosin stain of myometrium (left), with organized, normal-appearing smooth muscle cells, and fibroid tissue (right), with whorls of acellular extracellular matrix surrounding small clusters of disorganized smooth muscle cells.

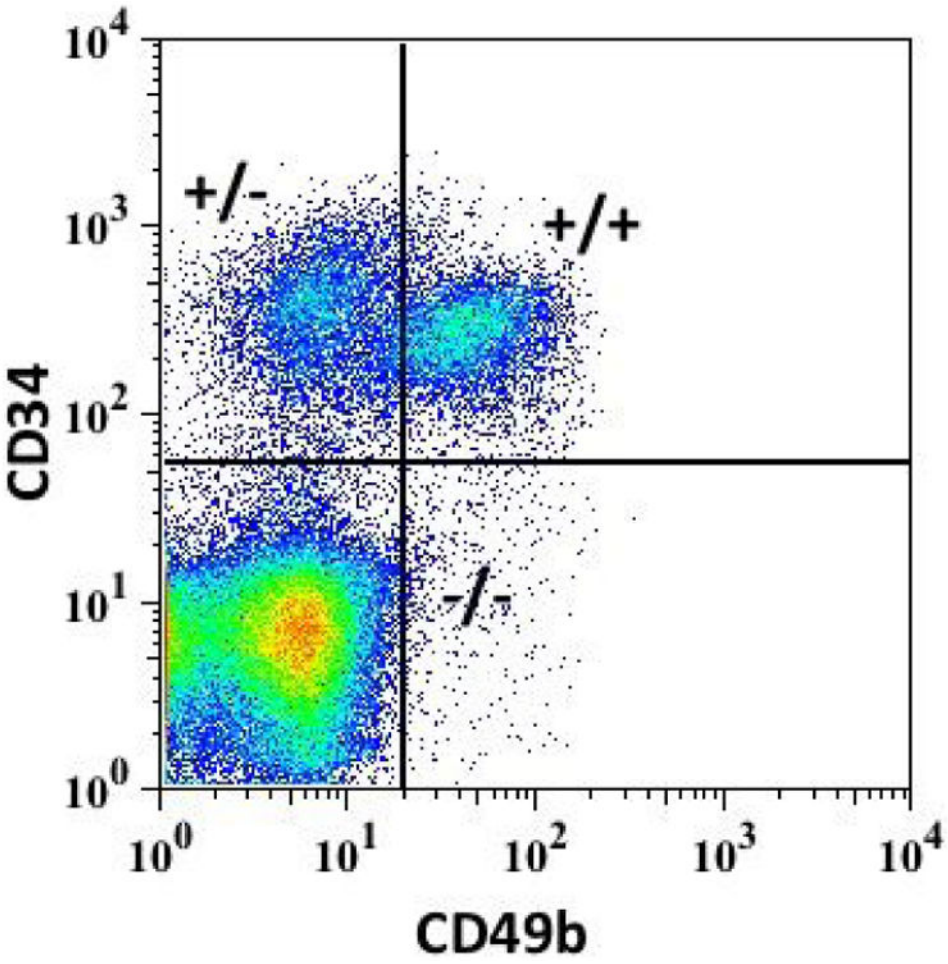

Figure 2.

Cell sorting by flow cytometry using antibodies to CD34 and CD49b revealed 3 distinct populations in fibroid cells: CD34+/CD34+ (+/+), CD34+/CD49b- (+/-), and CD34-/CD49b- (-/-). +/+ cells had characteristics of somatic stem cells, whereas -/- cells had a well-differentiated phenotype. We hypothesize that +/- are an intermediate cell type between +/+ and -/- cells.

Table 1

Currently available treatments for uterine fibroids[35-39].

Treatment Option	Route of Administration	Mechanism of Action	Potential Side Effects	Pregnancy Category	FDA Approved	Decreases Bleeding	Decreases Tumor Size	Additional Comments
GnRH agonists	Intramuscular, subcutaneous, or nasal spray	Abolishes GnRHpulsatility	Severe hypoestrogenemia: hot flashes, vaginal dryness, bone loss	X	Preoperative correction of anemia from fibroids	Yes	Yes	Initial flare effect; Requires add-back therapy after 6 months
GnRH Antagonists	Subcutaneous (Ganirelix), Oral (Elagolix)	Competitive inhibition of GnRH	Severe hypoestrogenemia: hot flashes, vaginal dryness, bone loss	X	No	Yes	Yes	Avoids flare effect of GnRH agonists
Selective Progesterone Receptor Modulators	Oral	Varied progesterone antagonism	Endometrial thickening/hyperplasia	X	No	Yes	Yes	Ulipristal approved for fibroid treatment in Europe and Canada
Aromatase Inhibitors	Oral	Competitive inhibition of aromatase	Bone loss	D	No	Yes	Yes	Can cause follicular stimulation
Oral Contraceptives	Oral	Stabilizes endometrium	Venous thromboembolism	X	Heavy menstrual bleeding	Yes	No	
Levonorgestrel-releasing IUD	Intrauterine	Induces endometrial atrophy	Breakthrough spotting, expulsion	X	Heavy menstrual bleeding	Yes	Conflicting data	Should not be used with intracavitary fibroids
Tranexamic acid	Oral	Inhibits Fibrinolysis	Fibroid infarction	B	Heavy menstrual bleeding from fibroids	Yes	No	Can be used for acute bleeding
Danazol	Oral	Synthetic androgen, inhibits steroidogenesis	Androgenic: voice changes, acne, hirsutism	X	No	Yes	No	High risk of side effects, use is generally discouraged