Vascular biology of uterine fibroids: connecting fibroids and vascular disorders

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Abstract

Fibroids are benign tumors caused by the proliferation of myometrial smooth muscle cells in the uterus that can lead to symptoms such as abdominal pain, constipation, urinary retention, and infertility. While traditionally thought of as a disease process intrinsic to the uterus, accumulating evidence suggests that fibroid growth may be linked with the systemic vasculature system, although cell-intrinsic factors are certainly of principal importance in their inception. Fibroids are associated with essential hypertension and preeclampsia, as well as atherosclerosis, for reasons that are becoming increasingly elucidated. Factors such as the renin-angiotensin-aldosterone system, estrogen, and endothelial dysfunction all likely play a role in fibroid pathogenesis. In this review, we lay out a framework for reconceptualizing fibroids as a systemic vascular disorder, and discuss how pharmaceutical agents and other interventions targeting the vasculature may aid in the novel treatment of fibroids.

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Introduction

Uterine leiomyomas (commonly referred to as ‘fibroids’) are the most common benign neoplasm of the female reproductive tract, present in up to 77% of reproductive-age women (Cramer & Patel 1990, Okolo 2008). While fibroids may be clinically silent, many women with fibroids suffer from heavy menstrual bleeding, chronic pelvic pain, dyspareunia, dysmenorrhea, bulk symptoms (e.g. urinary hesitancy, constipation), or pregnancy difficulties that negatively impact the quality of life (Zimmermann *et al.* 2012, Zepiridis *et al.* 2016). Indeed, symptomatic fibroids are responsible for over 350,000 hospitalizations and the leading indication for hysterectomy in the United States (Wechter *et al.* 2011, Shahid *et al.* 2020).

Despite the prevalence and burden of the disease, the origins and pathogenesis of fibroids remain largely a mystery. Clues to possible driving factors for the development and propagation of fibroids have come from their risk factors. Epidemiological studies have demonstrated that genetic factors such as positive family history and hypertension portend an increased risk of fibroids, suggesting that these factors may influence vascular dysfunction and play a role in fibroid pathogenesis (Radin *et al.* 2012, Wise & Laughlin-Tommaso 2016, Stewart *et al.* 2017).

Curiously, within the realm of hypertensive disorders, preeclampsia, a hypertensive and vascular disorder of pregnancy, has been positively associated with fibroid occurrence (Roberts *et al.* 1999, Pan *et al.* 2019). Further, polymorphisms in genes regulating vascular tone and arterial blood flow including the angiotensin-converting enzyme (ACE) and angiotensin receptor have been associated with fibroids, also suggesting a possible underlying pathological link (Gomaa *et al.* 2015, Keshavarzi *et al.* 2019).

It is of course worth acknowledging the progress that has been made in the fundamental understanding of fibroid biology. Fibroid development is a multistep process involving uterine smooth muscle stem cell alterations in estrogen, progesterone and Wnt signaling that lead to expansion of this cell population, followed by fibroblast and vascular smooth muscle cell recruitment and ECM deposition, leading to clinically significant fibroids (Stewart *et al.* 2016). Genetic changes causally related to the development of approximately 90% of fibroids include alterations in signaling involving the mediator subcomplex 12 (MED-12) and high mobility group protein (HMGA)1 and 2 genes. *MED12* is a highly conserved gene on the X chromosome that codes for a transcription factor regulating a multitude of genes pertinent to cell cycling/proliferation, including *WNT*, *CCND1*, *AXIN2*, and *MYC* (Makinen *et al.* 2011). While

MED12 mutation does not vary with patient age and race but does correlate with smaller tumors and more numerous tumors (He *et al.* 2021).

HMGA1 and the closely related *HMGA2* genes code for proteins involved in the regulation of chromatin architecture and transcription modulation, globally influencing cell cycle kinetics, differentiation and proliferation particularly of stem cell populations (Chieffi *et al.* 2002). Rearrangements leading to overexpression of *HMGA1* and *HMGA2* mRNA have been observed in fibroids, likely influencing tumor growth via direct promotion of proliferation and indirectly via upregulation of angiogenesis (Medeiros *et al.* 2007, Nezhad *et al.* 2010, Ferrero 2020, Li *et al.* 2020).

Apart from direct genetic mutations, epigenetic modifications and non-coding RNAs have been implicated in fibroid biology. Early life events, including environmental exposures in utero and in the prepubescent period (particularly to estrogens, genistein and diethylstilbestrol), likely alter epigenetic programs in uterine stem cell populations, reflected in differences in DNA methylation between fibroid cells and adjacent normal myometrium, predisposing to fibroid development later in life (Bulun 2013, Elkafas *et al.* 2017, McWilliams & Chennathukuzhi 2017). Further, alterations in miRNA, single-stranded non-coding RNA molecules, play a regulatory role in steroid-induced cellular processes important in fibroid biology via cytokine/chemokine mediators, including proliferation, apoptosis, ECM deposition, MMP activity, and autophagy (Bulun 2013, Ciavattini *et al.* 2013, McWilliams & Chennathukuzhi 2017, Ciebia *et al.* 2020).

Estradiol and progesterone are also synergistically involved in fibroid development. Estradiol stimulates estrogen receptors on uterine fibroblasts and promotes fibroblast proliferation and extracellular membrane production through fibroblast activation protein (FAP), MAP kinase activation, and activation of the PI3K-Akt-mTOR pathway *in vitro* (Luo *et al.* 2014, Borahay *et al.* 2017). Progesterone, on the other hand, activates progesterone receptors on fibroid cells which further activates the PI3K-Akt pathway, promoting fibroid cell survival *in vitro* (Hoekstra *et al.* 2009). In fact, inhibition of this pathway by gonadotropin receptor hormone (GnRH) therapy triggers fibroid cell apoptosis and is one modality of treatment for symptomatic fibroids (Yamada *et al.* 2004). On the other hand, combined oral contraceptive pills (OCPs), which contain estrogen and progestin, can reduce fibroid-related abnormal uterine bleeding, but they do so via a different mechanism, that is, by stabilizing and decidualizing the endometrium (Sayed *et al.* 2011).

In this article, we will refer to two related but distinct biological processes – vasculogenesis and angiogenesis. Vasculogenesis, or the *de novo* formation of blood vessels from pluripotent mesenchymal cells, is a developmental process that occurs in the uterus during

placentation which undergoes remodeling as gestation advances (Demir *et al.* 2006, Tal *et al.* 2019). Villous hemangiogenic cells develop into angioblasts which in turn differentiate into endothelial cells, initiating placental vasculogenesis (Demir *et al.* 2006). As opposed to vasculogenesis, angiogenesis is the sprouting of new vessels from preexisting vasculature. Both occur in the context of uterine biology and fibroid formation.

Given new developments in fibroid research, we lay out accumulating evidence in this article that fibroids may, at least in some circumstances, be part of a vascular disease process.

Overview of uterine vasculogenesis and angiogenesis

Once believed to occur solely through angiogenesis, uterine remodeling during pregnancy is now thought to also have a vasculogenic component (Tal *et al.* 2019). In pregnant mice, bone marrow-derived cells (BMDs), namely endothelial progenitor cells (EPCs), have indeed been shown to participate in decidual growth and uterine vasculogenesis, aside from that seen in placentation (Tal *et al.* 2019). From a pathological perspective, vasculogenesis is essential for tumor growth and maintenance, particularly when angiogenesis is blocked (Brown 2014). While vasculogenesis in uterine fibroids has yet to be definitively identified, these tumors have shown the capability to secrete CXCL12, a chemokine that recruits BMDs, perhaps facilitating fibroid growth through engrafting stem cells into the tumor (Moridi *et al.* 2020). Nevertheless, uterine vasculogenesis remains a largely unexplored avenue and whether BMDs contribute to uterine fibroid vasculogenesis *per se* remains to be understood.

Angiogenesis, by contrast, has long been known to occur in the uterus in physiological and pathological settings (Zygmunt *et al.* 2003). Given the dynamic nature of the endometrium and the increasing demand for blood supply throughout pregnancy, angiogenesis is deemed fundamental to sustain uterine physiology and establish fetal viability (Zygmunt *et al.* 2003, Demir *et al.* 2010). In addition to its contribution to the development of tumors at large, dysregulated angiogenesis has been reportedly noted in a wide array of uterine pathologies, including placenta accreta spectrum, abnormal uterine bleeding, as well as the focus of this review, uterine fibroids (Jaffe 2000, Tseng & Chou 2006, Tal & Segars 2014). Uterine fibroids have a well-vascularized capsule yet a hypovascular core, a unique demarcation that has led the scientists to propose the presence of an inherent angiogenic defect (Carmeliet 2003, Tal & Segars 2014). Our knowledge of the exact culprits behind these observations is limited and further understanding of the possible mechanisms that underlie the vascular pathobiology of uterine fibroids is crucial.

Fibroid composition and vasculature

Fibroid composition

Fibroids are characterized by benign monoclonal proliferation of smooth muscle cells and fibroblasts, with extensive deposition of extracellular matrix components (Holdsworth-Carson *et al.* 2014). The clonality of these tumors implies an internal (oncogenic) driver of proliferation. At the same time, it is possible that vascular changes may modulate this cell-intrinsic propensity of monoclonal proliferation leading to the initiation and growth of fibroids, as will be discussed further later. Fibroids are differentiated from leiomyosarcoma (malignant proliferation of smooth muscle cells) and from smooth muscle tumors of uncertain malignant potential (STUMP lesions) based on the mitotic index, degree of cellular atypia and necrosis (Watanabe & Suzuki 2006, Bacanakgil *et al.* 2017). Fibroids have been posited to arise from an aberrant myometrial proliferative/extracellular matrix (ECM) synthetic reparative response to vascular injury or ischemia (Flake *et al.* 2013). On an ultrastructural level, fibroid cells exhibit increased protein synthesis machinery (rough endoplasmic reticulum, free ribosomes, and Golgi apparatus) and contain fewer contractile elements as compared to normal myometrial cells (Flake *et al.* 2013).

Aside from their basic make-up, fibroids are dynamic in structure and activity in the early stage of their life cycle, contrary to what might be supposed based on static histological images. Indeed, fibroid development can be divided into four phases: differentiated based on collagen content (which increases as fibroids mature), myocyte proliferation (active in phases 1–3), collagen synthesis (active in phases 2 and 3), and involution (which has the highest collagen content) (Flake *et al.* 2013).

Vascular supply

Fibroids depend almost entirely upon the uterine arteries for recruitment of their blood supply, although the uterus additionally receives secondary blood supply from the ovarian arteries, which could theoretically feed a proximal fibroid. Fibroids develop a rich network of secondary branches and disrupt and enlarge the caliber and course of the uterine arteries (Pelage *et al.* 2005). Likewise, the uterine arteries of fibroid uteri have increased blood flow, lower resistance index and pulsatility index as compared to the uterine arteries from non-fibroid uteri (Kurjak *et al.* 1992). For instance, fibroids display ongoing blood flow in both systole and diastole, as well as a higher flow rate compared to the main uterine arteries, demonstrating a relentless demand for blood supply (Kurjak *et al.* 1992). Consistent with this finding, uterine blood flow rate also correlates positively with fibroid size (Alatas *et al.* 1997). By contrast, fibroids themselves are quite hypovascular at their cores and are supplied by small peripheral arteries via arterial plexuses, with few containing intrafibroid

arteries (Pelage *et al.* 2005, Idowu & Ibitoye 2018). The type of cell death that fibroids exhibit in response to lack of blood supply has been referred to as 'inanosis,' or death due to nutritional deprivation, as fibroids typically lack the histological hallmarks of either necrosis or apoptosis (Flake *et al.* 2013). The vascular smooth muscle cells of vessels supplying the fibroids likewise can exhibit parallel features to the fibroid cells themselves. Thus, while fibroids constitute the product of monoclonal proliferation of a neoplastic cell, implying a cell-intrinsic process, the propensity of such cells to develop and grow into a fibroid may be modulated by vascular factors such as blood flow patterns and vascular-derived signals.

Clinical correlates

Hypertension

The association between uterine fibroids and hypertension has been extensively addressed in the literature. Several studies have found a significant positive correlation between the two disorders (Luoto *et al.* 2001, Haan *et al.* 2015, 2018), but causality has not yet been successfully established. A prospective study has examined the relation between diastolic blood pressure and the incidence of clinically detected uterine fibroids and detected a 10 and 8% rise in fibroid risk for each 10 mmHg increase in diastolic blood pressure among users and nonusers of antihypertensives, respectively (Boynton-Jarrett *et al.* 2005). In considering the relationship between hypertension and fibroids, it is important to consider potential confounding factors. In fact, one study found that the association between hypertension and fibroids persisted even after adjusting for obesity, African ancestry, hormonal contraceptive use, parity, postmenopausal status, fasting plasma cholesterol and fasting plasma glucose (except for diabetes), with odds ratios between 1.80 and 1.90 (Haan *et al.* 2018).

On the other hand, hypertension results in smooth muscle cell injury through mechanical shear stress, perhaps involving the uterine vasculature that induces myomatous proliferation and fibroid genesis (Fig. 1) (Humphrey 2008). Elevated blood pressure also induces proinflammatory *milieus* and upregulates ECM synthesis, in part through the action of TGF- β , which has been seen to activate fibroblasts in rats induced to have hypertension (Kuwahara *et al.* 2002, Boynton-Jarrett *et al.* 2005). Another mediator possibly implicated in the association between hypertension and uterine fibroids is the enzyme creatine kinase (CK) (Hoag *et al.* 1980, Brewster *et al.* 2006), which has been shown to induce vascular and uterine smooth muscle cell proliferation by replenishing ATP for vascular contractility and trophic processes (Karamat *et al.* 2014, Haan *et al.* 2018). Indeed, higher levels of CK have been detected in leiomyomatous tissues compared to healthy myometrium (Hoag *et al.* 1980). The consistent evidence of the correlation between hypertension and uterine fibroids

Figure 1 Schematic presentation of the association of uterine fibroids with systemic vascular disorders. (1) Similar to preeclampsia, the association of uterine fibroids with hypertension may be mediated by ET-1. (2) RAA system activation and hypertension-mediated smooth muscle injury and ECM upregulation may explain the association between uterine fibroids and hypertension. (3). Uterine fibroids and atherosclerosis are resemblant in terms of pathological features and associated risk factors. Angiotensin, Ang; endothelin-1, ET-1; extracellular matrix, ECM; renin-angiotensin-aldosterone, RAA; transforming growth factor β , TGF- β .

raises the prospect, once again, of vascular involvement in fibroid pathobiology. Although speculations largely predominate the underlying pathophysiology of this association, uterine fibroids may now be perceived as a marker for hypertension and that women with fibroids may start to be screened for elevated blood pressure and vice versa (AlAshqar *et al.* 2019). Not only this approach may expedite the detection of a largely asymptomatic disease such as hypertension but also decrease the risk of cardiovascular outcomes in the long run (Laughlin-Tommaso *et al.* 2019).

Atherosclerosis

An accumulating body of evidence has supported the notion that uterine fibroids and atherosclerosis bear pathophysiological resemblance (Fig. 1). This hypothesis is reinforced by several observations: (1) both atherosclerosis and uterine fibroids represent a pathological proliferative lesion of smooth muscle cells; (2) similar to fibroids, atheromatous plaques may have a monoclonal origin; and (3) both lesions can eventually fibrose and calcify (Benditt & Benditt 1973, Moss & Benditt 1975). In each case, the production of reactive oxygen species (ROS) leads to stimulation of fibroid cell or vascular smooth muscle cells by platelet-derived growth factor (PDGF), leading to enhanced the MAP kinase mitogenic signaling, stimulating smooth muscle cell proliferation (Shimizu *et al.* 2009, Mesquita *et al.* 2010). From a clinical perspective, while some studies have failed to detect an association between subclinical cardiovascular disease and uterine fibroids (Laughlin-Tommaso *et al.* 2019), others demonstrated that indicators of atherosclerosis are more prevalent among women with uterine fibroids. For instance, a population-based longitudinal study found an independent positive association between fibroids and LDL and triglyceride levels (Uimari *et al.* 2016). As stated previously, Aksoy

et al. concluded that cIMT, a surrogate marker for early atherosclerosis, is significantly elevated in women with fibroids, which may allow for its use as a screening measure for subclinical cardiovascular disease in this population (Aksoy *et al.* 2014).

Moreover, a case-control study of Chinese women investigated the association of atherogenic risk factors with uterine fibroids and concluded that higher ankle-brachial index (ABI) readings, another marker of early atherosclerosis, are associated with increased odds of uterine fibroids. Our group has also previously reviewed the various mechanisms underlying the association between uterine fibroids and cardiometabolic risk factors (Fig. 1), lending further support to the presence of shared pathophysiology between fibroids and atherosclerosis (AlAshqar *et al.* 2019). In fact, cardiometabolic risk factors, such as advanced age and hypertension, have been shown to significantly correlate with uterine artery atherosclerosis (Crawford *et al.* 1997), but whether the latter may serve as a marker for atherosclerosis in other vascular beds or is a potential culprit in fibroid initiation and growth is yet to be investigated. Nevertheless, our growing knowledge of the association between atherosclerotic vascular disease and uterine fibroids will pave our way to a better understanding of the vascular involvement in uterine fibroids.

Preeclampsia

Although scarce, evidence of an association between uterine fibroids and preeclampsia has been reported in the literature. A case-control study by Pan *et al.* has documented uterine fibroids as a significant pre-existing medical risk factor for preeclampsia (Pan *et al.* 2019). Further, a study by Roberts *et al.* concluded that multiple fibroids imply a higher risk of preeclampsia compared to a single fibroid, which the authors attributed to the disruption of trophoblastic invasion of spiral arteries caused

by the expanding fibroid (Roberts *et al.* 1999). Although plausible, there may be more to the role of fibroids in preeclampsia occurrence than a mere mechanical effect. As previously discussed, mediators, such as ET-1, ghrelin, and sHLA-G, may represent common grounds on which vascular dysfunction may contribute to uterine fibroids and preeclampsia and where research efforts can be therefore guided in the future.

Intriguingly, uterine natural killer (uNK) cell involvement may be a potential mechanism that links uterine fibroids to preeclampsia. For example, altered numbers of uNK cells in the endometrium of women with uterine fibroid has been hypothesized to decrease fertility and promote aberrant vascular development, possibly leading to higher risk of recurrent miscarriages and implantation failure (Quenby *et al.* 2009, Lash & Bulmer 2011). On the other hand, studies quantifying decidual uNK cells in preeclamptic women have shown mixed and inconsistent results that may be better overcome by conducting the analysis during pregnancy than after delivery (Bachmayer *et al.* 2006, Lash & Bulmer 2011). Nevertheless, given the increasingly recognized role of uNK in spiral artery remodeling and vascular wall invasion by the extravillous trophoblast (Lash & Bulmer 2011), the notion that aberrant uNK cell numbers may contribute to preeclampsia is still valid and a hypothesis that can be further investigated.

Potential biomarkers implicated in the vascular pathobiology of uterine fibroids

A plethora of circulating markers have been reported in the literature to have a differential expression in women with and without uterine fibroids, a few of which may be potentially involved in the vascular aberrancies seen in these tumors.

Prolactin

Prolactin has been demonstrated to be expressed by uterine endometrial and myometrial tissues and to a greater extent by uterine fibroids (Walters *et al.* 1983, Daly *et al.* 1984, Rein *et al.* 1990). It remains unclear as to whether fibroid-secreted prolactin contributes to the development of the tumor itself or is rather a mere marker of tumor growth. However, it was shown that the addition of prolactin-neutralizing antibodies to myometrial and fibroid cell cultures halts cellular proliferation, suggesting that prolactin may indeed behave as a growth factor in an autocrine or paracrine fashion (Nowak *et al.* 1999). In addition, prolactin has been found to have mitogenic properties in vascular smooth muscle cells through a protein kinase C-mediated mechanism, predisposing to vascular smooth muscle cell hyperplasia, a feature reminiscent of what happens in hypertension and atherosclerosis (Sauro & Zorn 1991). These findings lend further support to the

vascular theory of fibroid pathogenesis and may link their occurrence to systemic vascular disorders. Although evidence suggests that elevated serum prolactin levels are observed in patients with uterine fibroids (Baban 2009), other common causes of hyperprolactinemia including the presence of an asymptomatic prolactinoma, were not taken into account. Hence, while such results may show promise in the potential use of prolactin as a fibroid marker, further experimental studies are needed to validate this observation.

Ghrelin

Ghrelin, a peptide hormone involved in energy regulation, is another marker that has been found to be associated with uterine fibroids. A study has concluded that patients with symptomatic fibroids have significantly higher active ghrelin and active to total ghrelin ratio compared to controls matched for BMI. However, as levels largely overlap between cases and controls, the use of ghrelin as a biomarker may not be possible (Markowska *et al.* 2009). In addition, although it was shown that ghrelin is produced and processed in the human myometrium (O'Brien *et al.* 2010), its role in uterine fibroids *per se* is yet to be elucidated. Within the context of vascular dysfunction, serum ghrelin level has been positively correlated with the severity of preeclampsia but negatively so with uterine artery Doppler index values and systolic and diastolic blood pressures (Erol *et al.* 2016). Although these observations may seem conflicting, a few mechanisms have been suggested to explain the increase in serum ghrelin in preeclampsia, possibly explaining its increased levels in uterine fibroids. Given the body of evidence that suggests a protective effect of ghrelin against hypertension, atherosclerosis, and cardiovascular disease (Zhang *et al.* 2010), the increase in ghrelin in preeclamptic women may be a compensatory mechanism to derangements in some mediators involved in the disease (Aydin *et al.* 2008, Erol *et al.* 2016). In addition, ghrelin may manifest inhibitory actions during early pregnancy that interfere with proper embryo implantation on the one hand (Delgado & Ganea 2008) and angiogenic actions that induce aberrant vascular remodeling on the other hand, both of which may increase the risk of preeclampsia (Zaniolo *et al.* 2011, Erol *et al.* 2016). Nevertheless, these mechanisms are largely speculative and further *in vivo* and *in vitro* studies are needed to confirm these speculations.

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF), a master regulator of neovascularization, has been found to be elevated in patients with uterine fibroids compared to those without, with levels declining after hysterectomy (Chen *et al.* 2005). This comes in line with previous work showing the uterus' capability of VEGF production

(Torry *et al.* 1996, Agrawal *et al.* 2000), but this also suggests that VEGF expression may be more pronounced in uterine pathology, including fibroids. Whereas some studies detected higher VEGF expression in fibroids compared to normal myometrium (Hague *et al.* 2000, Gentry *et al.* 2001), others concluded no differential expression of VEGF mRNA and VEGF protein between myometrial and fibroid smooth muscle cells (Harrison-Woolrych *et al.* 1995), emphasizing the need for more consistent data in this regard. Despite the lack of evidence of its growth-promoting actions in smooth muscle cells (Ferrara *et al.* 1992), VEGF may contribute to uterine fibroids in a mechanism that is not necessarily mitogenic. VEGF stimulates angiogenesis and increases vascular permeability, potentially facilitating tumor growth, and induces endothelial cell proliferation, indirectly upregulating other growth factors. For example, VEGF can liberate basic fibroblast growth factor (bFGF) from moieties in the extracellular matrix, which in turn has mitogenic properties in smooth muscle cells (Jonca *et al.* 1997, Flake *et al.* 2003). Despite the interest in this growth factor as a potential culprit of abnormal vascular function in uterine fibroids, it may not serve as an effective biomarker as it does not predict fibroid development (Chen *et al.* 2005).

Hematopoietic growth factors

Hematopoietic growth factors (HGFs), namely macrophage-colony stimulating factor (M-CSF) and granulocyte-colony stimulating factor (G-CSF), have been evaluated as potential candidate markers for uterine fibroids. Both markers are increased in patients with uterine fibroids compared to controls and can be used to distinguish between endometrial cancer and fibroids, particularly when used with CA125 (Lawicki *et al.* 2010). While HGFs may not have direct mitogenic actions on smooth muscle cells, they have been shown to promote tumor angiogenesis (Ribatti & Tamma 2019). Indeed, HGFs have been demonstrated to induce endothelial proliferation and migration both *in vivo* and *in vitro* (Bussolino *et al.* 1989, 1991). In contrast, endothelial cells exposed to VEGF manifested increased release of HGFs by bone marrow endothelial cells (Bautz *et al.* 2000), highlighting a reciprocal association between HGFs and key angiogenic factors that may or may not occur on pathophysiological grounds in uterine fibroids.

Tumor necrosis factor α

A growing body of evidence now shows that tumor necrosis factor α (TNF- α) may play a particularly notable role in the development of uterine fibroids. *In vitro* studies have demonstrated increased proliferation of human fibroid cells and expression of anti-apoptotic markers when exposed to TNF- α . In addition, this pro-proliferative effect has been reversed upon adding anti-TNF- α

antibodies (Nair & Al-Hendy 2011). Further, women with uterine fibroids are found to have an approximately two-fold increase in serum TNF- α levels compared to controls, with levels increasing with fibroid size (Ciebia *et al.* 2018a). These findings may suggest the potential use of TNF- α as a marker for uterine fibroids, using a level cut-off point that may also help to distinguish fibroids from STUMP lesions and leiomyosarcoma (Ciebia *et al.* 2018b). Although the actions of TNF- α in fibroids appear to be tumorigenic, the literature has reported a role for TNF- α in vascular dysfunction elsewhere. For instance, TNF- α was shown to downregulate the production of nitric oxide (NO) via inhibition of endothelial NO synthase (eNOS), thereby impairing smooth muscle relaxation and inducing endothelial dysfunction (Zhang *et al.* 2009). This was further supported by the finding that TNF- α predisposes to endothelial dysfunction in animal models with diabetes and metabolic syndrome by means of reactive oxygen species production (Picchi *et al.* 2006, Lee *et al.* 2017a). Thus, given the existing evidence that uterine fibroids may be associated with cardiometabolic risk factors and atherosclerosis (AlAshqar *et al.* 2019), inflammation may contribute to vascular dysfunction in fibroids in a similar way to that seen in cardiometabolic risk factors.

Soluble HLA G

Soluble HLA G (sHLA-G), a human non-classical major histocompatibility complex molecule involved in immune regulation and expressed in the uterus, has been found to be statistically significantly higher in women with uterine fibroids compared to controls (Basta *et al.* 2009, Levy *et al.* 2013). In addition, it was found to be elevated in patients with systemic sclerosis, an autoimmune disorder with profound vascular involvement (Contini *et al.* 2018). Although not entirely understood, sHLA-G was suggested to contribute to systemic sclerosis pathogenesis through its interaction with transforming growth factor β (TGFB1) (Contini *et al.* 2018). It is well-established that TGB1 plays a significant role in the pathobiology of uterine fibroids through downstream signaling with Smad and the PI3K-AKT-mTOR and MEK-ERK pathways (Ciebia *et al.* 2017) but whether sHLA-G interacts with TGFB1 or has any pathogenic role in uterine fibroids has yet to be determined. From another perspective, it is worth noting that sHLA-G serves to promote proinflammatory cytokine secretion in the placenta, facilitating trophoblast migration and vascular remodeling (Dahl *et al.* 2014). Although controversial, studies linked HLA-G genotypes and aberrant HLA-G protein expression with preeclampsia, with most studies showing reduced serum levels in preeclamptic women (Hara *et al.* 1996, Dahl *et al.* 2014). This points to the potential involvement of sHLA-G in vascular pathology, but further investigation is warranted regarding its role in uterine fibroids.

Lactate dehydrogenase A

Lactate dehydrogenase A (LDHA) is another blood marker that exhibits significantly higher levels in patients with uterine fibroids vs those without (Koukourakis *et al.* 2009). However, as LDHA can also be elevated in patients with uterine sarcoma and endometrial cancer (Song *et al.* 2018), LDHA is difficult to interpret in patients with uterine masses, rendering it unhelpful as a fibroid biomarker (Koukourakis *et al.* 2009). Although its role in uterine fibroid development, if any, remains unexplored, LDHA has been implicated in vascular dysfunction. Upregulation of LDHA and glycolysis in vascular smooth muscle cells, stimulated by platelet-derived growth factor (PDGF), promotes the proliferation and migration of these cells, perhaps predisposing to atherosclerosis (Kim *et al.* 2017). This observation may link the development of uterine fibroids to an element of local and systemic vascular dysfunction, but further experimental validation is needed.

Cancer antigen 125

Cancer antigen 125 (CA125), a marker best known for its association with ovarian cancer, can, in fact, be elevated in a variety of gynecologic and nongynecological disorders that produce peritoneal irritation, including, among many other entities, uterine fibroids (Juang *et al.* 2006, Levy *et al.* 2013). It is largely unknown whether CA125 is actively involved in the pathogenesis of uterine fibroids or is passively secreted by the tumor instead. Nevertheless, a link between CA125 and vascular dysfunction has been reported in the literature. In patients with epithelial ovarian cancer, for example, intracystic VEGF levels were positively correlated with serum CA125 levels. This was hypothesized to occur due to VEGF-mediated enhancement of tumor angiogenesis and vascular permeability that leads to increased spillage of CA125 into the circulation (Candido Dos Reis *et al.* 2002). Although this may not suggest a pathogenic role for CA125 in uterine fibroids, it may point to similar vascular processes in the fibroid that may parallel the increase in serum CA125. The implications of CA125 in vascular pathology extend beyond gynecologic perspectives as its serum levels have been found to be correlated with carotid intima-media thickness (cIMT) in patients with coronary artery disease (Sang *et al.* 2018). Intriguingly, cIMT has been found to be significantly higher in women with uterine fibroids compared to those without, suggesting that fibroids may indeed share common etiopathogenic mechanisms with atherosclerotic vascular disease (Aksoy *et al.* 2014, AlAshqar *et al.* 2019). Although inflammation seems to underlie, at least in part, the increase in CA125 and vascular dysfunction (Sang *et al.* 2018), further evidence is needed to confirm the nature of this association.

Molecular biology of abnormal vascular function in fibroids

Endothelin-1 (ET-1) and soluble Fms-like tyrosine kinase-1 (sFlt-1)

The hypovascularity of fibroids creates a hypoxic microenvironment that serves as a positive feedback loop driving proliferation and neovascularization (Fig. 2). For instance, endothelin-1 (ET-1) is a potent vasoconstrictor peptide secreted by endothelial cells and fibroid smooth muscle cells in response to hypoxia. ET-1 is elevated in the plasma of women with fibroids, compared to those without, and drives fibroid cell proliferation (Wallace *et al.* 2014, 2018). Blockade of ET_A receptors on fibroid smooth muscle cells under both normoxic as well as hypoxic conditions leads to decreased proliferation of these cells, suggesting an autocrine function for ET-1 that helps drive fibroid growth (Wallace *et al.* 2018). Beyond promotion of fibroid cell division, ET-1 has been shown to have a direct angiogenic effect on endothelial cells and indirect effects via stimulation of vascular endothelial growth factor (VEGF) (Knowles *et al.* 2005). Intriguingly, ET-1 dysregulation has also been implicated in preeclampsia (Fig. 1). It has been postulated that abnormal placentation in the uterine wall in patients who will go on to develop preeclampsia leads to elevated circulating levels of anti-angiogenic factors such as soluble Fms-like tyrosine kinase-1 (sFlt-1), a VEGF decoy receptor that functionally inactivates VEGF and causes a rise in ET-1, likely through tissue hypoxia (Saleh *et al.* 2016). Indeed, sFlt-1 binds VEGF with ten-fold greater affinity than native receptors, making sFlt-1 a VEGF sink and effectively enhancing ET-1 synthesis in response to hypoxia (Wallace *et al.* 2014). Circulating levels of sFlt-1 in women with fibroids are increased by nearly 50% as compared to those without, suggesting the pathophysiological significance of this small molecule *in vivo* (Wallace *et al.* 2014).

Elevated ET-1, in turn, drives systemic vasoconstriction, vascular dysfunction, and elevated mean arterial pressure, all characteristics of preeclampsia (Possomato-Vieira & Khalil 2016). The fact that fibroids are also associated with elevated ET-1 levels and systemic hypertension suggests a possible unifying pathogenic link between these two disease entities that can be traced to abnormal uterine vascularity. Further evidence supporting this common underlying pathophysiology is the intriguing clinical association shown in several studies that smoking may be paradoxically protective against both fibroids and preeclampsia. It is puzzling that smoking seems to be directly related to atherosclerosis but inversely related to fibroid development. This can perhaps be partially explained through nicotinic effects on the balance of angiogenic factors, although this phenomenon remains controversial and poorly understood (Baron 1996, Parazzini *et al.* 1996, England & Zhang 2007, Templeman *et al.* 2009,

Figure 2 Schematic presentation of vascular dysfunction and vasoactive mediators involved in uterine fibroid pathogenesis. (1) Aberrant activation of the RAA system is implicated in fibroid cell proliferation through the actions of Ang-II and aldosterone. (2) The tumorigenic actions of Ang-II are potentiated via ACE and ATR gene polymorphisms whereas those of aldosterone may be mediated by MAPKs and ERKs. (3) Estrogen can augment the actions of the RAA system in fibroids by upregulating hepatic angiotensinogen production and AT₂R expression in the uterine vasculature. (4) Ang-II may additionally upregulate TGFβ1 expression and ECM synthesis. (5) Vascular dysfunction in uterine fibroids may create a local hypoxic milieu that upregulates HIF-1α, and in turn, ET-1 expression. In the fibroid cell, ET-1 activates the enzyme SphK1, promoting the conversion of sphingosine to S1P, which inhibits apoptotic cascades and enhances cell survival. (6) Increased basal NO induces ROS production, inhibition of apoptosis, endothelial dysfunction, and systemic vascular disorders. (7) Collectively, increased cellular proliferation, decreased apoptosis and enhanced ECM synthesis promote fibroid growth. Dashed lines denote hypothesized mechanisms. Angiotensin, Ang; angiotensin-converting enzyme, ACE; angiotensin receptor, ATR; endothelin-1, ET-1; extracellular matrix, ECM; extracellular regulated kinases, ERKs; gonadotrophin-releasing hormone, GnRH; hypoxia-inducible factor 1α, HIF-1α; mineralocorticoid receptor, MR; mitogen-activated protein kinases, MAPKs; nitric oxide, NO; reactive oxygen species, ROS; renin-angiotensin-aldosterone, RAA; sphingosine kinase 1, SphK1; sphingosine-1-phosphate, S1P; transforming growth factor β, TGF-β.

Karumanchi & Levine 2010). Another possibility to explain this epidemiological observation is the moderating effect of smoking on estrogen levels. It has been established that smoking is associated with lower circulating levels of estradiol (vs not smoking), likely contributing to related elevated risks such as osteoporosis and fragility fractures in smokers as compared to nonsmokers (Brook *et al.* 2012, Wong *et al.* 2016). Circulating estradiol levels have also been found to correlate positively with the risk of fibroid development, although this correlation is modulated by testosterone and dehydroepiandrosterone sulfate (DHEAS) levels (Wong *et al.* 2016). Thus, it stands to reason that the 'protective' effect of smoking on fibroid development may be due, at least in part, to a reduction in circulating estrogen, which normally acts to drive fibroid growth, although this remains to be directly demonstrated.

Renin-angiotensin-aldosterone (RAA) system

Another physiological vascular circuit that fibroids appear to 'hijack' to promote their growth is the renin-angiotensin-aldosterone (RAA) system (Fig. 2). The RAA system is an evolutionarily conserved endocrinological feedback circuit involving the kidneys, lungs, liver, and vasculature that acts to increase mean arterial pressure in response to a perceived drop in circulatory volume. When the kidneys sense decreased perfusion, they release renin, an enzyme that cleaves liver-derived angiotensinogen to angiotensin-I (Ang-I), which is then converted to the bioactive ligand angiotensin-II (Ang-II) via the angiotensin-converting enzyme (ACE) in the lung. Ang-II has direct vasoconstrictive effects on smooth muscle cells of the vasculature and stimulates the adrenal glands to produce aldosterone, a hormone

that promotes renal tubular reabsorption of water and sodium to maintain adequate circulatory volume. Excessive activation of this pathway is thought to play a key role in the development of essential hypertension, and drugs that block components of these pathways (e.g. ACE inhibitors, ACE-I, or angiotensin receptor blockers, ARBs) are cornerstones in the treatment of essential hypertension (Te Riet *et al.* 2015).

Notably, aside from vascular smooth muscle cells, fibroid cells express angiotensin receptors (ATRs), and stimulation of these receptors by the ligand Ang-II induces fibroid cell proliferation, an effect that is abolished by ARBs (Isobe *et al.* 2008). However, whether circulating levels of Ang-II differ between patients with and without fibroids remains unknown. Extrapolating from the hypertension literature, there may not be dramatic differences in the levels of circulating Ang-II, but rather fibroid cells may be particularly sensitive to small fluctuations in local concentrations of Ang-II, perhaps due to polymorphisms in *ACE* or *ATR* genes or due to greater exposure to Ang-II over time given their blood supply (Nussberger *et al.* 1989, Henry *et al.* 2020). Indeed, polymorphisms in the *ACE* gene (specifically the ID and DD genotypes) have been associated with an elevated risk of fibroid (as compared to the II genotype), although whether there exists a causal link remains controversial (Gultekin *et al.* 2015, Keshavarzi *et al.* 2019). Of note, the angiotensin-II receptor 1 (*AT₁R*) A1166C gene polymorphism, which leads to increased *AT₁R* protein expression and has been associated with endothelial dysfunction and increased left ventricular mass, was not found to be associated with fibroid risk in a case-control study, although this is only one of a host of possible genetic variants in this receptor (Jin *et al.* 2012, Li *et al.* 2015, Keshavarzi *et al.* 2019). Other pathological mutations in various component proteins in the RAA system remain to be uncovered, and possible changes in Ang-II in patients with and without fibroids remain to be empirically tested.

Finally, the RAA system contains a counterbalancing arm of the circuit that may act as negative feedback on the fibroid growth. The type-2 angiotensin-converting enzyme (*ACE2*) generates angiotensin 1–7 (Ang 1–7), which is a functional antagonist of Ang-II (Casalechi *et al.* 2018). Ang 1–7 binds to the Mas receptor, a G protein-coupled receptor, which is present in human endometrium, and whose stimulation has antifibrotic and anti-proliferative downstream effects (Casalechi *et al.* 2018). Given the anatomical distribution, the Ang 1–7-Mas receptor pathway may be particularly relevant to the growth of submucosal fibroids or may be relevant to fibroid growth more generally through paracrine signaling to the myometrial tissue.

Estrogen, progesterone, and pregnancy

On the other hand, there is reason to suspect that Ang-II levels may play a role in fibroid growth through the

effects of estrogen. Estrogen potently activates fibroblast proliferation in fibroids, and high estrogen states have been associated with increased fibroid size, while medications that block estrogen's production/activity can induce fibroid shrinkage (Donnez *et al.* 1989, Borahay *et al.* 2017). These effects have been attributed to direct effects of estrogen acting on fibroid cell estrogen receptors. However, estrogen also regulates transcription of angiotensinogen in the liver (Fig. 2), and thus may indirectly induce fibroid growth through upregulation of the Ang-II precursor (Gordon *et al.* 1992, Krattenmacher *et al.* 1994). Furthermore, high estrogenic states, such as pregnancy, induce both increased Ang-II production and increased expression of *AT₂R* on the uterine arteries, which lead to a paradoxical increase in uterine blood flow (given Ang-II's normal vasoconstrictive effect). These effects are directly mediated through elevated estradiol levels (Mishra *et al.* 2018).

In addition to changes in estrogen that occur with pregnancy, progesterone elevation in pregnancy may also contribute to the regulation of fibroid growth. For instance, one study of women with fibroids undergoing *in vitro* fertilization (IVF) with luteal phase support with 200 mg intravaginal progesterone found that fibroids underwent a phase of rapid growth during early pregnancy, increasing by as much as 35% in diameter 4–5 weeks after embryo transfer, as compared to pre-pregnancy (Benaglia *et al.* 2014). While the action of several hormones or their interplay could explain this phenomenon, this rapid fibroid growth correlating with a rise in progesterone, either locally or systemically, that occurs starting in the first trimester, could partially contribute 10902789 (Luisi *et al.* 2000). In any case, progesterone in the experimental setting has been shown to be important for the maintenance phase of fibroid growth, an effect that is potentiated by estradiol-induced expression of progesterone receptors on fibroid cells and abrogated by the progesterone receptor blocker mifepristone (Ishikawa *et al.* 2010).

Fibroids' shunting of blood supply to meet their growing needs is multipronged, involving not only increased factors that promote angiogenesis/vasculogenesis but also likely via increased production of red blood cells. Case reports have described polycythemia in patients with large fibroid burden, so-called 'myomatous polycythemia,' that often resolves after myomectomy (Fig. 1), suggesting that fibroids may also recruit increased blood supply through stimulation of bone marrow production of red blood cells (Hertko 1963, Takkar & Kumar 1994, Abdul Ghaffar *et al.* 2008). Possible underlying mechanisms include mass effect on the thorax leading to chronic hypoventilation or fibroid-induced vascular dysfunction leading to hypercoagulability and chronic thromboembolic disease, which can stimulate erythropoietin production (Vanden Berg & Vasu 1963, Lacharite-Roberge *et al.* 2019). Again, this phenomenon is not entirely understood, and thus further research of

novel mechanisms in which fibroids likely represent a systemic vascular disorder is necessitated.

Contribution of altered vascular biology to fibroid growth

How do all of the factors described previously lead to the growth of fibroids on a cellular level? First, we will examine how ET-1 can produce such an effect (Fig. 1). ET-1 inhibits apoptosis in multiple cell types, including fibroid cells, through its interaction with the sphingosine-1-phosphate (S1P) pathway. S1P is a bioactive sphingolipid metabolite that regulates a host of physiological and pathological processes including, pertinent to this discussion, atherosclerosis, inflammation, and tumor growth (Maceyka *et al.* 2012). Serum-starved rat fibroid cells (ELT3 cells) initiate apoptotic programming via the release of cytochrome C from mitochondria, caspase-3/7 activation, and DNA fragmentation (Raymond *et al.* 2006). The addition of ET-1 is sufficient to block this apoptosis cascade by activating sphingosine kinase 1 (SphK1), the enzyme that catalyzes the conversion of sphingosine to the bioactive form S1P (Raymond *et al.* 2006). Moreover, ET-1 contributes to vascular inflammation and remodeling through its interaction with hypoxia-induced factor-1 α (HIF-1 α) (Gras *et al.* 2016). Whether the severely hypoxic microenvironment characteristic of fibroids induces a classic HIF-1 α response (i.e. leading to activation of HIF-1 α response elements in the nucleus and target gene transcription) remains controversial, with several studies finding no changes in HIF-1 α expression in fibroids (although the HIF-1 system is robustly activated in leiomyosarcoma (Sadri & Zhang 2013)). The increased HIF-1 α expression in fibroid cells may nevertheless lead to upregulation of several downstream genes involved in angiogenesis, including VEGFA (Mayer *et al.* 2008, 2010, Ishikawa *et al.* 2019). Whether ET-1 modulates the HIF-1 α pathway in response to tumor hypoxia in the case of fibroids, perhaps explaining this discrepancy, remains to be further understood.

With regard to the RAA system, Ang-II and aldosterone likely both play a direct role in stimulating fibroid growth through various intracellular signaling pathways (Fig. 1), although these effects have been more extensively studied in the cardiovascular field as compared to the fibroid field. In terms of aldosterone, fibroid cells do express mineralocorticoid receptors (MRs) and are responsive to aldosterone stimulation (Isobe *et al.* 2010). For instance, incubation of rat fibroid cells with aldosterone leads to a dose-dependent increase in proliferation, an effect abolished by the MR antagonists spironolactone and eplerenone (Isobe *et al.* 2010). In terms of intracellular signaling cascades that may be activated downstream of MRs in fibroid cells remains to be determined, but extrapolating from renal mesangial cells, it is possible that the mitogen-activated protein kinases (MAPKs) and

extracellular signaling-related kinases 1/2 (ERK1/2) are involved (Nishiyama *et al.* 2005).

In terms of Ang-II-induced changes in fibroid cellular physiology, the current state of evidence comes from a mix of cardiovascular literature and fibroid literature, as direct results from fibroids/uterine tissue are still incomplete. In cardiac myocytes, for instance, the autocrine release of Ang-II leads to load-induced hypertrophy via transforming factor β (TGF- β) (Sadoshima *et al.* 1993, Schultz Jel *et al.* 2002). TGF- β , through intracellular signaling involving the SMAD protein complex, is known to play important roles not only in cell growth and survival but also in fibrosis through upregulation of extracellular matrix (ECM) deposition and remodeling (Massague & Wotton 2000, Schiller *et al.* 2004, Biernacka *et al.* 2011). Indeed, TGF- β expression is markedly increased in fibroid smooth muscle cells as compared to normal myometrial cells and is largely responsible for orchestrating the weaving of collagens into the connective tissue that characterizes the fibroid composition (Zeyneloglu *et al.* 2008, Joseph *et al.* 2010, Borahay *et al.* 2015a). In addition to promoting ECM deposition, TGF- β may also interfere with the expression of ECM-degrading genes such as matrix metalloproteinases (MMPs) (Bogusiewicz *et al.* 2007, Joseph *et al.* 2010).

Is the elevated TGF- β signaling in fibroids restricted to the fibroid bed, or might this process be far-reaching? A recent investigation found that patients with fibroids exhibit elevated serum levels of TGF- β , which may serve as a biomarker for fibroid disease severity, in addition to potentially risk stratifying which women with fibroids may go on to develop systemic vascular complications (Kamalipooya *et al.* 2020). This finding is consistent with the notion that systemic dysregulation of TGF- β signaling underlies a host of fibrotic diseases involving the vasculature, such as scleroderma, glomerulonephritis, and subclinical atherosclerosis (Malik *et al.* 2010, He *et al.* 2013). For instance, a cross-sectional case-control study found that carotid artery intima-media thickness was significantly elevated in patients with fibroids vs those without (Aksoy *et al.* 2014). Further, antifibrotics have been used in the experimental setting to inhibit collagen production and fibroid cell proliferation with some success, though the effects of such antifibrotics on the development of vascular disorders in patients with fibroids has not been studied (Grudzien *et al.* 2010).

Nitric oxide (NO) signaling and endothelial dysfunction

Nitric oxide is a free radical produced from L-arginine via nitric oxide synthase (NOS) that participates in a host of physiological processes including vasodilation and inhibition of platelet aggregation (Oh *et al.* 2013). Expression of eNOS in the endometrium and myometrium of patients with fibroids is significantly elevated as

compared to those of control women, suggesting upregulation of this signaling pathway in fibroid uteri (Oh *et al.* 2013). Likewise, baseline NO production is elevated in culture media from fibroid cells vs that of normal myometrial cells (Favini *et al.* 2003). Functionally, this translates into greater oxidative stress, an effect that is exacerbated by the hypoxic microenvironment of fibroids (Fletcher *et al.* 2017). This increased free radical production leads to increased protein nitration and nitrosylation, which inhibits pro-apoptotic pathways and has been proposed as a means by which fibroid cells escape apoptosis (Fig. 1) (Fletcher *et al.* 2017).

How does aberrant NO signaling then lead to endothelial dysfunction? In general, endothelial dysfunction is characterized by proinflammatory, prothrombotic properties and a decreased propensity toward vasodilation (Endemann & Schiffrin 2004). Vasoactive peptides such as Ang-II, ET-1, and NO modulators may contribute to this dysfunction, which can result in atherogenesis, endothelial cell apoptosis and sloughing (anoikis) (Endemann & Schiffrin 2004). In the context of fibroids, NO-mediated oxidative stress and related endothelial dysfunction may contribute to the development of systemic hypertension and atherosclerosis in these patients (Fig. 1) (He *et al.* 2013, Uimari *et al.* 2016, Fletcher *et al.* 2017). In agreement, antioxidants that theoretically quench these excess free radicals have shown some clinical benefit in terms of decreasing fibroid volume, although this remains to be further explored and validated (Roshdy *et al.* 2013).

In summary of this section, fibroids interact extensively with the vasculature to promote their growth. In doing so, they borrow signaling pathways that work not only to regulate their local physiology but also bi-directionally with the systemic circulation, potentially promoting systemic hypertension, vascular dysfunction, and fibrosis.

Approaches to treatment of fibroids that target the vasculature

In the previous section, we explored various ways in which fibroids interact with the vasculature to promote their growth and the molecular underpinnings of these interactions. Given the demonstrated effects of fibroids on the uterine and systemic vasculature, it stands to reason that treatments targeting the vasculature may also present viable options to address fibroids.

Hormonal modalities

Traditional approaches to treating fibroids can be divided into medical vs surgical methods. Medical options have come in the form of hormonal regulation of the hypothalamic-pituitary-ovarian (HPO) axis. Hormonal management of fibroids has typically been

conceptualized as directly affecting fibroid growth through estrogen and progesterone receptors on fibroid cells. For instance, combined oral contraceptive pills (OCPs), which contain formulations of estradiol and a progestin, as well as progestin-containing intrauterine devices, have been mainstays in the treatment of fibroid-related abnormal uterine bleeding, with the levonorgestrel-containing IUD showing a slightly greater reduction in fibroid-related menstrual blood loss (Sayed *et al.* 2011).

Gonadotropin-releasing hormone (GnRH) agonists have also been shown to lead to fibroid shrinkage through suppression of the HPO axis via continuous receptor stimulation. Similarly, selective progesterone receptor modulators through downstream intracellular G-protein-coupled receptor signaling additionally lead to fibroid shrinkage. However, these treatments are generally limited to the preoperative setting before myomectomy or hysterectomy in order to avoid long-term side effects associated with their use (Lee *et al.* 2017b). Interestingly, GnRH agonists act not only to suppress the HPO axis, but relevant to the current discussion, also decrease blood flow to the uterus. This may be mediated not only through effects on estrogen and progesterone receptors on fibroid cells but also through targeting of AT₂R expression on the uterine arteries (Fig. 1) (Mishra *et al.* 2018). This may help to explain the decreased blood loss and less need for blood transfusion associated with myomectomy in patients who receive preoperative GnRH agonists vs those who do not (Lethaby *et al.* 2017).

Targeting RAAS

The theoretical benefit of decreasing Ang-II signaling to treat fibroids was recently addressed in a nested case-control trial of women with essential hypertension. Women were divided according to whether or not they had been regularly taking an ACE-I, with the outcome of clinically recognized fibroid development across a 5-year period (Fischer *et al.* 2020). Women who had been taking an ACE-I for their hypertension had 31.8% reduced odds of subsequent fibroid development vs those not taking an ACE-I, lending credence to the idea that inhibition of the RAA system may be a novel prophylactic strategy to prevent fibroids in at-risk women. In this study, comorbidities including heart and lung disease, renal failure, liver disease, cancer, obesity, alcohol use disorder, collagen vascular diseases, and psychiatric disorders were adjusted in order to isolate the association between ACE-I use and odds of fibroid. Individual ACE-Is were studied, and several different ACE-Is were found to be associated with significantly decreased odds of fibroids, including lisinopril, quinapril, and ramipril. This study opens a line of investigation into many intriguing clinical and

mechanistic questions. For instance, whether ACE-I use in women with pre-existing fibroids leads to fibroid shrinkage or decreased symptomatology remains to be studied. Additionally, whether antihypertensives that do not specifically target the RAAS but lower blood pressure via alternative mechanisms remains unclear. Our lab is actively working on this area of investigation, and there will likely be answers to these and other related questions in the coming years.

Statins

Apart from hormonal manipulation and RAAS targeting to decrease fibroid vascular flow and size, other strategies targeting the vasculature include the use of HMG-CoA reductase inhibitors (i.e. statins). Statins act in a pleiotropic fashion by competitively blocking the rate-limiting enzyme in cholesterol synthesis (HMG-CoA reductase), preventing the conversion of HMG-CoA to mevalonic acid, and thereby lowering low-density lipoprotein cholesterol (LDL-c), and are the mainstay of treatment of dyslipidemia that can induce atherosclerosis (Ward *et al.* 2019). Statins act not only to decrease the accumulation of plaque on the systemic arteries by blocking LDL-c production but also have anti-inflammatory properties and may improve endothelial dysfunction (Pretnar-Oblak *et al.* 2008, Taqueti & Ridker 2017). Indeed, statin use is associated with a reduction in ROS and downregulation of AT1R in leukocytes of subjects with dyslipidemia (Guasti *et al.* 2008). A nested case-control study showed that exposure to statins within 2 years was associated with a significantly decreased odds of developing fibroids, as well as a lower likelihood of developing heavy menstrual bleeding, anemia, or pelvic pain (Borahay *et al.* 2016). Additional work in cell culture and animal models has demonstrated that statins cause direct caspase-3/Bim/Bcl2 mediated apoptosis and inhibition of ERK1/2, JNK, and Akt signaling in fibroid cells, similar to their effects on vascular smooth muscle cells, promoting apoptosis and stunting growth, respectively (Fig. 1) (Borahay *et al.* 2014, 2015b, Shen *et al.* 2018). Furthermore, statins were found to inhibit the deposition of excessive extracellular matrix proteins and ameliorate the altered mechanotransduction in fibroids (Malik *et al.* 2018, Afrin *et al.* 2020). Could statins also inhibit fibroid growth through effects on the uterine arteries or fibroid vascular bed? Uterine artery dysfunction occurs in the setting of an experimental model of dyslipidemia, raising the possibility that statins may also act to normalize fibroid uterine blood flow, perhaps contributing to their shrinkage or degeneration, although this remains to be tested (Taylor *et al.* 2003).

Non-pharmacological modalities

Finally, a non-pharmacological vascular approach to treating fibroids, short of removing the fibroids

or the uterus surgically, is known as uterine artery embolization (UAE). In this procedure, the systemic arterial circulation is entered via the common femoral artery. A microcatheter is introduced, and the path of the vasculature is traced back to the uterine arteries. At this point, small (500–1000 μm diameter) polyvinyl alcohol or tris-acyl gelatin particles are fed into the uterine arteries to occlude arterial flow (Keung *et al.* 2018). Remarkably, uterine artery blood flow normalizes following successful UAE with shrinkage of fibroids, as measured by Doppler flow, further suggesting that fibroids act as intrinsic blood siphons within the uterus (McLucas *et al.* 2002).

Thus, both pharmacological and non-pharmacological treatment modalities that involve either the systemic or the local uterine vasculature have come to light as viable options for patients with fibroids. While much of this work remains experimental or preliminary, it is likely that we will see increasing efforts to decrease or reverse fibroid growth through vessel-targeting mechanisms. Conversely, it will be interesting to determine whether treatment of fibroids can also promote improvements in other associated vascular disorders such as hypertension, preeclampsia, atherosclerosis or various vasculitides.

Conclusion

Fibroids have been extensively studied over the past few decades, with significant strides made in terms of basic pathophysiology and cellular signaling. Through this work, our understanding of fibroid growth and development has become enriched, and it is increasingly clear that fibroids do not exist as an isolated entity distinct from the functioning of the rest of the human body. Indeed, in clinical studies, fibroids are often found in association with systemic diseases, especially those involving endothelial or vascular dysfunction. This newly recognized link raises interesting fundamental questions about possible causality and whether fibroids represent a uterine manifestation of a systemic disease process. Here, we have laid out the current evidence supporting the notion that fibroids are intrinsically linked to vascular biology and may in fact contribute to vascular dysfunction. This has important implications regarding the long-term health prognosis of women who have fibroids and also introduces a novel framework with which to begin to address the treatment of fibroids. It will be exciting to see how further advances in the understanding of fibroid physiology evolve over time and allow clinicians to better treat these mysterious but common benign gynecological tumors.

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G W K and A A A wrote the initial draft of the manuscript. M M-I, L R, M E S and M A B edited the draft. All authors read, edited, and approved the final draft.

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