Matrix production and remodeling as therapeutic targets for uterine leiomyoma

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Abstract Uterine leiomyoma, commonly known as fibroids, is a benign neoplasm of smooth muscle in women. The incidence of clinically symptomatic fibroids in reproductive-age women is approximately 20 %, with nearly 80 % of black women suffering from this condition. Symptoms include severe pain and hemorrhage; fibroids are also a major cause of infertility or sub-fertility in women. Uterine leiomyoma consist of hyperplastic smooth muscle cells and an excess deposition of extracellular matrix, specifically collagen, fibronectin, and sulfated proteoglycans. Extracellular matrix components interact and signal through integrin- β 1 on the surface of uterine leiomyoma smooth muscle cells, provide growth factor storage, and act as co-receptors for growth factor-receptor binding. ECM and growth factor signaling through integrin- β 1 and growth factor receptors significantly increases cell proliferation and ECM deposition in uterine leiomyoma. Growth factors TGF- β , IGF, PDGF, FGF and EGF are all shown to promote uterine leiomyoma progression and signal through multiple pathways to increase the expression of genes encoding matrix or matrix-modifying proteins. Decreasing integrin expression, reducing growth factor action and inhibiting ECM action on uterine leiomyoma smooth muscle cells are important opportunities to treat uterine leiomyoma without use of the current surgical procedures. Both natural compounds and chemicals are shown to decrease fibrosis and uterine leiomyoma progression, but further analysis is needed

to make inroads in treating this common women's health issue.

Keywords Fibroids · Infertility · Myometrium · Reproductive biology · Smooth muscle

Abbreviations

AKAP	A-kinase anchor protein
Bcl	Burkitt cell lymphoma
CDK	cell division kinase
ECM	extracellular matrix
EGF	epidermal growth factor
Erk	extracellular signal-regulated kinase
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
HSPG	heparan sulfate proteoglycan
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
MAPK	mitosis-activating protein kinase
MMP	matrix metalloprotease
PDGF	platelet-derived growth factor
SB	<i>Scutellaria barbata</i> D. Don
SMC	smooth muscle cells
TGF	transforming growth factor
UAE	uterine artery embolization
UL	uterine leiomyoma

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Background

Uterine leiomyoma are benign tumors of uterine myometrium and are more commonly known as uterine fibroids. Uterine leiomyoma (UL) are prevalent among reproductive-aged and post-menopausal women, and afflict a large proportion of women in the United States. The cumulative incidence for

leiomyoma in the female African American population is estimated to be 65 % by the age of 35 and 80 % for black women over the age of 50. Caucasians have a decreased incidence; however the rates are still high, with 20 % of women having symptomatic fibroid tumors by the age of 35 (Baird et al. 2003).

In a prospective cohort study of premenopausal women 15–44, African American women were over three times more likely to have a diagnosed uterine leiomyoma than a Caucasian woman (Cardozo et al. 2012). Interestingly, the study also showed that physical activity and null-parity are not shown to significantly influence the incidence of UL in premenopausal women (Cardozo et al. 2012). There is currently little evidence that particular lifestyle choices contribute to UL incidence; however, the majority of UL are shown to be chromosomally normal, suggesting that external factors at the hormonal and cellular level are most likely contributing to this disparity and to the high incidence in women of reproductive age (Levy et al. 2012).

Uterine leiomyoma are often associated with significant bleeding and pain, which have a negative impact on quality of life for those women afflicted. Heavy and prolonged bleeding, frequent periods, irregular periods, pain during sexual intercourse, pain throughout the menstrual cycle, and bladder pressure are all symptoms commonly identified in women with uterine fibroids (Zimmerman et al. 2012). Although not every woman with uterine fibroids has severe symptom, current treatments are limited and there are substantial costs each year associated with treating ULs.

Women who suffer from uterine fibroids often require treatment that can significantly affect the fertility in women of reproductive age. As a result, UL are a significant healthcare burden in the United States, with an estimated \$9 billion going towards annual direct costs to cover surgeries, hospital admissions, outpatient visits and medications (Marshall et al. 1997). Lost work-hour costs were estimated to be \$17 billion. In total, it is estimated that uterine fibroids cost the United States >\$30 billion annually (Marshall et al. 1997).

The exact cause of UL is not yet known, although leiomyoma growth is known to be associated with exposure to ovarian hormones estrogen and progesterone. Women who are burdened with UL during pregnancy often show an increase in the growth of their fibroids (Takeuchi et al. 2000), presumably due to increased exposure to estrogen and/or progesterone, yet it is worth noting that there is no conclusive evidence supporting this assumption. An increase in leiomyoma growth is observed during the secretory phase of the menstrual cycle, and as in pregnancy, this is often interpreted as a result of the spike in both estrogen and progesterone (Lamminen et al. 1992). However, it is progesterone production that dominates the secretory phase, while relatively high estrogen levels have been present for more than

2 weeks during the proliferative phase and remain high during the secretory phase. Since the increase in fibroid size is seen in the secretory phase but not in the proliferative phase, it is highly likely that progesterone is the key component in this process, a notion that is corroborated by a considerable amount of evidence in cell culture and animal models.

Interestingly, xenoestrogens have been shown to influence the incidence of leiomyomas. Xenoestrogens are chemicals that biochemically mimic estrogen by binding to estrogen receptors upon ingestion; therefore, leiomyoma prevalence is not only shown to be associated with an increased level of endogenous estrogen, but also exposure to an increased level of xenoestrogens (Shen et al. 2013). However, xenoestrogens are also thought to cause increased body weight, which will alter steroid hormone production, so it is not clear if the xenoestrogen effect is direct or indirect. Furthermore, it is possible that the xenoestrogens may be partial or weak agonists for the progesterone receptor; this possibility remains to be investigated before xenoestrogens could be considered a key player in the pathogenesis of fibroids.

Despite studies exploring hormonal contribution, much evidence indicates that there are other important pathways promoting UL growth. A study by Lobel et al. found that levels of estrogen and progesterone are similar in African American and Caucasian women, despite the increased frequency, earlier age of onset, number of fibroids per uterus, and larger size of UL in African American women (Lobel et al. 2006). Although estrogen and progesterone were shown to promote UL growth, the major differences in the above parameters without a significant difference in hormone levels warrant further investigation into other pathways contributing to UL growth and formation (Lobel et al. 2006).

Like most benign tumors, uncontrolled cell proliferation contributes to the size and growth of UL; however, aberrant cell proliferation in uterine leiomyoma contributes only partially to the volume and size of the fibroid. As the name “fibroid” suggests, uterine fibroids have the distinctive features of fibrosis. A hallmark of uterine leiomyoma is the excessive deposition of extracellular matrix (ECM) (Malik et al. 2012). The ECM components contributing to the majority of the volume in UL are: proteoglycans, fibronectin and collagen (Ingber and Folkman 1989). As with other tissues in the body, the ECM action in UL stimulates cell-cell adhesion, cell migration, cell proliferation and cell differentiation and promotes further ECM deposition (Ingber and Folkman 1989).

Due to the large contribution of ECM to the volume and growth of UL, this review focuses on the interaction between the extracellular environment and uterine smooth muscle cells (SMC). We highlight studies that reveal how these interactions contribute to cell proliferation and increased ECM production through cell signaling and increased gene expression. Though there is a dearth of research on non-surgical therapies to

alleviate UL in reproductive-age women, this review will conclude with a review of current therapies, as well as potential therapeutic targets to reduce cell proliferation and ECM deposition in fibroids

Components of extracellular matrix in uterine leiomyoma

As previously mentioned, the hallmark of ULs is the increased deposition of extracellular matrix. Preliminary research shows the greatest proportion of ECM between uterine leiomyoma smooth muscles cells is composed of collagen, fibronectin and proteoglycans.

Collagen content in uterine leiomyoma Like most tissues in the body, the ECM in uterine tissue, whether normal myometrium or a fibroid, contains a substantial amount of collagen. Collagen is the most abundant protein in the body and collagen expression is shown to increase in various tissues that undergo fibrosis, including lung, liver and bone marrow (Karsenty and Park 1995). Several studies confirm similar findings in UL, and show that the excess ECM in uterine fibroids is due to both an overproduction of collagen and a decrease in collagen breakdown. One study showed collagen deposition is a significantly greater proportion of the extracellular matrix composition in UL when compared to healthy uterine tissue (Leppert et al. 2004). Another group examined collagen deposition in UL by measuring levels of prolyl hydroxylase, an enzyme that catalyzes a critical step—the production of hydroxyproline from proline—during post-translational modification of the collagen protein. The study found that prolyl hydroxylase expression is significantly increased in UL when compared to normal myometrium throughout the menstrual cycle, suggesting that the increased production of collagen contributes to the excess ECM found in UL (Iwahashi et al. 2012).

Several studies show that collagen type 1 gene expression is significantly greater than the gene expression of other collagen types, suggesting collagen type 1 is the predominant collagen component in uterine leiomyoma ECM. These studies also found that collagen type 1 expression is significantly increased in leiomyoma SMC when compared to normal uterine myometrium (Carrino et al. 2012; Iwahashi et al. 2011).

Recent studies suggest that, in addition to contributing to the fibrosis and volume of fibroids, collagen may also play a role in SMC proliferation in UL. Koohestani et al. examined fibroid SMC plated on monomeric collagen and found a significant increase in cell proliferation and cell cycle transit times. These increases were significantly greater when compared to normal myometrial SMC plated on the same collagen substrate (Koohestani et al. 2013). Additionally, there

appeared to be a good correlation between the amount of ECM deposition, the concentration of growth factors in the ECM, the fraction of proliferating cells, and the size of the fibroid.

Fibronectin content in uterine leiomyoma Fibronectin is a matricellular protein that plays an integral role in mediating cell-cell interactions, cell adhesion, and cell proliferation (Pankov and Yamada 2002). Fibronectin exists as a dimer and binds to collagen type I in the ECM of tissues throughout the body (Pankov and Yamada 2002). Fibronectin exists as a dimer and binds to collagen type I in the ECM of tissues throughout the body (Dzamba et al. 1993). The binding of fibronectin with collagen type I in the ECM provides a strong scaffold structure for cell adhesion, and also participates directly in cell signaling.

Immunohistochemical staining of cultured UL SMC showed a significantly greater expression of fibronectin compared to cultured myometrial SMC (Kobayashi et al. 1996). The overproduction and secretion of fibronectin in UL is influenced by hormones, growth factors and is also shown to be regulated by collagen type I expression in the ECM (Moore et al. 2010; Dzamba et al. 1993; Halder et al. 2011). Fibronectin and collagen type I form a scaffold that promotes the excessive fibrosis in uterine leiomyoma, and growth factor binding sites on fibronectin provides a growth factor storage source in the ECM (Lin et al. 2011). This growth factor storage allows for increased cell signaling when the ECM is degraded and the released growth factor proteins are able to bind receptors on the SMCs. This growth factor action will be discussed in more detail later in this review.

Proteoglycans content in uterine leiomyoma Glycosaminoglycans (GAGs) are polysaccharide chains of amino sugars that play an important role in extracellular matrix structure, composition and function. Some GAGs covalently bind to a core protein to form proteoglycans; these include heparan sulfate, chondroitin sulfate, and keratan sulfate. Dermatan sulfate and hyaluronan are GAGs that do not bind to a core protein, but still contribute significantly to the ECM composition and function (Esko et al. 2009). GAGs and proteoglycans have the ability to bind ligands, such as integrins and growth factor receptors, in the extracellular environment. They often act as facilitators of ligand-ligand binding, serve as a co-receptor for growth factors, and also create a repository for growth factors in the extracellular matrix (Esko et al. 2009). GAGs and proteoglycans, together with collagen, provide the structural basis for the extracellular matrix in uterine leiomyoma, and appear to play an important role in fibroid formation and growth (Esko et al. 2009).

Past studies have identified differences in GAGs between fibroid and normal myometrial SMC. A recent study comparing the wet weights of GAGs from the ECM of leiomyoma and myometrial tissue found that GAG weight in fibroids is significantly greater than that of normal uterine tissue (Wolanska et al. 1998). Other studies also found that sulfated glycosaminoglycans, including heparan sulfate, keratan sulfate, chondroitin sulfate and heparin, are the predominant GAGs in both uterine fibroids and normal myometrium. Sulfated GAGs are shown to have a greater binding capacity for certain growth hormones than unsulfated GAGs (Esko et al. 2009).

Another group identified differences in the types of GAGs in the ECM between UL and normal myometrium. Samples of normal myometrium and leiomyomas were taken from the same patient, and six different patient specimens were included in the study. Using gel chromatography, Mitropoulou et al. were able to isolate five sulfated and non-sulfated GAGs from both tissues, including heparan sulfate, keratan sulfate, chondroitin sulfate, dermatan sulfate, and hyaluronan. The study found that dermatan and keratan sulfates are significantly greater in leiomyoma tissue compared to myometrium, and heparan sulfate is significantly decreased in UL (Mitropoulou et al. 2001).

The upregulation of dermatan and keratan sulfate in UL ECM suggests enzymes that target and degrade these GAGs could be used as therapies in reducing the ECM deposition. These GAGs may also store particular growth factors that act on leiomyoma SMC, thus providing insights into which growth factor pathways could be targeted in therapy.

Extracellular matrix component influence on uterine leiomyoma progression

ECM influence on the physical characteristics of fibroids The increased deposition of collagen, fibronectin and glycosaminoglycans contributes to changes in the physical characteristics of leiomyoma tissue compared to that of healthy myometrium. UL have the ability to retain water and solutes in the network of GAGs present in the extracellular matrix (Kobayashi et al. 1996). Studies have shown the orientation and organization of collagen in the uterine leiomyoma interstitial matrix is abnormal, which creates the firm and rigid characteristic of uterine leiomyoma (Tomasek et al. 2002).

Normal myometrial cells subjected to applied strain forces are known to reorient their cytoskeleton parallel to the direction of the applied strain; however, in a recent study a significantly smaller proportion of leiomyoma cells reoriented under the same mechanical stimulation (Tomasek et al. 2002). These findings support a previous study observing collagen fibrils to be more loosely packed and antiparallel to applied

strain in fibroid SMC compared to the parallel, more tightly packed ECM of normal myometrium (Leppert et al. 2004).

The reduced orientation of leiomyoma SMC, combined with the increased density of ECM components, creates an environment that provides a strong scaffold for ECM components and smooth muscle cell integrins to interact (Tomasek et al. 2002). Studies have shown that the increase in rigidity and density of the ECM can lead to an increase in cell proliferation through mechanically-induced gene expression signals and is a characteristic of tumorigenesis (Norian et al. 2012; Butcher et al. 2009). Their evidence suggests that the mechanical induction is through integrin-ECM interactions leading to downstream signaling within fibroid SMC.

Integrin-ECM extracellular to intracellular signaling (Fig. 1) Integrins are heterodimeric transmembrane proteins capable of signaling across cell membranes through interactions with ECM components and through intracellular signaling cascades. Integrins also play an important role in tissue repair, serving as membrane receptors involved in cell-cell adhesion and anchoring cells to the extracellular matrix. Integrin $\beta 1$ is one of the most prevalent β subunits in the integrin family. When activated, integrin $\beta 1$ contributes to cell proliferation, tumor invasiveness, and cell survival in various tissues (Malik et al. 2012; Weaver et al. 1997). Integrin $\beta 1$ has

Fig. 1 Collagen type 1 extracellular induction of intracellular cell progression and proliferation signals through AKAP13, RhoA, and MAPK/Erk1/2

been implicated in a number of pathologies, including both benign and malignant cancers (Weaver et al. 1997). (Fig. 2)

There is growing evidence that integrin $\beta 1$ contributes to increased cell proliferation in UL via increased cell-to-ECM contact. A recent study showed that integrin $\beta 1$ expression was significantly upregulated in fibroid SMC (Chen et al. 2013). These investigators used Western blot analysis to confirm that UL SMC had a significantly greater expression of integrin $\beta 1$ protein compared to normal myometrial SMC. Increased expression of integrin $\beta 1$ suggests that fibroid SMC may have more opportunities to bind ECM via integrin $\beta 1$ (Chen et al. 2013). A study by Malik et al. found similar results, observing a 2.3 fold increase in expression of integrin $\beta 1$ on the surface of leiomyoma SMC compared to the surface expression on healthy myometrium. They concluded the increased expression of integrin $\beta 1$ and the increased signaling through ECM-integrin $\beta 1$ may impair the cellular response to signals due to over-stimulation. This may contribute to the inability of fibroid SMC to reorient the cytoskeleton parallel to applied strain (Malik et al. 2012).

Chen et al. also compared uterine leiomyoma tissue with integrin $\beta 1$ knockout UL tissue to examine potential differences in the interaction of SMC with the extracellular matrix (Chen et al. 2013). They observed diminished cell spreading and adhesion in integrin $\beta 1$ -knockout tissues. Furthermore, they showed that cyclin D1 expression was significantly decreased in integrin $\beta 1$ -knockout cells, leading to slowed cell cycle progression and decreased cell proliferation compared to fibroid SMC with normal integrin $\beta 1$ expression. The results of Malik et al. supported the findings by Chen et al. and strongly suggests an important role for integrin $\beta 1$ in regulating fibroid SMC proliferation rates.

Previous studies have demonstrated that integrins connect intracellular actin focal adhesion plaques to the ECM (DeMali et al. 2003; Iwahashi et al. 2012). Integrin-collagen type 1 interactions are known to activate intracellular signaling through focal adhesions in uterine leiomyoma (Carrino et al. 2012). One study showed a dose-dependent relationship of integrin $\beta 1$ expression and the levels of collagen type 1 in the extracellular environment. The mechanical strain on collagen

Fig. 2 Growth factor association with the ECM plays an integral role in cell cycle progression, cell proliferation and ECM deposition in UL SMC. Here MMP-2 degrades several ECM components, releasing growth

factors to bind to their receptors. Several ECM components modulate growth factor binding to the respective receptor, such as HSPG with FGF, fibronectin with PDGF, and IGF to several ECM components

may induce signaling through integrins, leading to stimulation of various downstream signaling pathways (DeMali et al. 2003).

Integrin activation leads to a number of intracellular signaling pathways in various cell types. The intracellular tails of integrins interact with actin cytoskeleton filaments of focal adhesions. A recent study by Rogers et al. used fluorescent electron microscopy to show that A-kinase anchor protein 13 (AKAP13) is associated with the structural filaments of the actin cytoskeleton in uterine leiomyoma smooth muscle cells and in myometrium (Rogers et al. 2008). AKAP13 is a guanine nucleotide exchange factor that activates GTPases, initiating an intracellular signaling cascade in UL SMCs. The study also showed that AKAP13 expression is significantly increased in uterine leiomyoma cells compared to myometrium.

Studies have identified RhoA as a downstream effector of AKAP13. AKAP13 is shown to activate RhoA from the inactivated form to the activated RhoA GTPase (Park et al. 2002; Shibolet et al. 2007). This finding is also shown to be true in fibrotic tissues. A very recent study showed that AKAP13 activation of RhoA acts as a pro-fibrotic signal in cardiac muscle tissue (Cavin et al. 2014). Malik et al. conducted a study comparing active RhoA between normal myometrium and leiomyoma tissue. RhoA is a GTPase known to take part in regulating cell division and is shown to promote cell proliferation and growth of tissues in various cell types. The study found that not only is RhoA activation significantly higher in serum-treated leiomyoma compared to myometrium, but also antibody-mediated inhibition of integrin $\beta 1$ led to a decrease in active RhoA, whereas the level of active RhoA was unaffected in normal myometrium (Malik et al. 2012; DeMali et al. 2003). Inhibition of integrin $\beta 1$ could be an important therapeutic target if signaling and integrity of normal tissue is unaffected while signaling in UL SMCs is halted.

The same laboratory conducted a study to investigate the downstream signals of integrin $\beta 1$ and to determine differences in RhoA activation. Norian et al. showed that in addition to leiomyoma tissue being more sensitive to integrin $\beta 1$ activation through RhoA, basal levels of active RhoA and its downstream effector, Rho-kinase (ROCK), are significantly higher in leiomyoma smooth muscles cells compared to healthy myometrium (Norian et al. 2012). The RhoA/ROCK pathway may provide an opportunity for therapies targeting overexpressed integrin $\beta 1$ in UL.

Malik et al. show that extracellular-signal regulated kinase (Erk) is a downstream effector of integrin-dependent RhoA activation, by measuring phosphorylated Erk levels before and after integrin-antibody inhibition in leiomyoma smooth muscle cells. p-Erk was significantly reduced after treatment with an integrin $\beta 1$ inhibitor (Malik et al. 2012). Studies in different cells show that mitogen-activated protein/Erk

(MAPK/Erk) activation leads to an increase in gene expression of cyclins, transcriptional regulators, collagens, and proteins involved in cell cycle progression, proliferation and survival (Chaturvedi et al. 2007).

Studies in pulmonary epithelial cells suggest that mechanically induced activation of integrin-ECM signaling increases cell proliferation and promotes cell survival. These studies also suggest that the integrin-ECM interactions signal through MAPK/Erk activation (Chaturvedi et al. 2007; Yu et al. 2012). A recent study demonstrates that integrin $\beta 1$ activation of MAPK/Erk increases expression of proteins and enzymes that play an integral role in cell cycle progression and cell proliferation, specifically cyclin D1 and cyclin-dependent kinase 2 (CDK2) (Wang et al. 2011; Taylor et al. 1996). The increase in expression of cyclin D1 and CDK2 in this study supports the aforementioned studies showing an integrin-induced increase in cell proliferation and cell cycle progression; however, further investigation is needed to confirm integrin $\beta 1$ is signaling through MAPK/Erk in uterine leiomyoma (Taylor et al. 1996).

Other integrins have been identified as playing a role in UL growth compared to integrin expression in normal myometrium. Among these are integrin $\alpha 1$, $\alpha 3$, $\alpha 4$, $\alpha 5$, αv , $\alpha 6$, and $\beta 3$ (Taylor et al. 1996). These integrins are receptors for many extracellular matrix proteins, including fibronectin ($\alpha 3\beta 1$, $\alpha 4\beta 1$, $\alpha 5\beta 1$, $\alpha v\beta 1$, $\alpha v\beta 3$) and collagen ($\alpha 1\beta 1$, $\alpha 3\beta 1$) (42). Currently, there is little known about the many other integrins that have been described in uterine leiomyoma, despite the large number of these heterodimeric receptors that have been shown to functionally interact with ECM proteins in many different tissues.

A recent study by Tsai investigated integrin αv involvement in cell survival and signaling in UL. Signaling through integrin αv decreased cell survival and increased apoptosis in normal myometrium. The study also found that the expression of integrin αv was significantly reduced in fibroids compared to myometrium. Integrin αv signaling in healthy myometrium suggests that the decreased expression of integrin αv in UL is contributing to the sustained growth of the tumor (Tsai et al. 2011). This intriguing observation illustrates the complexity of cell signaling, cell survival, and apoptosis in the pathogenesis of UL.

Relationship between ECM and growth factors, and their action on UL

In a 2004 review, Mueller and Fusenig presented a cyclical pattern in tumor progression in the interactions between tumor cells and their surrounding interstitial matrix. Their analysis found that crosstalk between tumor cells and ECM relies heavily on the constant deposition and decomposition of growth factors and ECM components. They proposed a cyclical pattern based on several studies, including one by Sato

et al., which studied uterine cervical carcinoma cells co-cultured with uterine cervical fibroblasts. This study found that both cell lines secreted increased amounts of matrix metalloproteinases (MMPs), enzymes responsible for the degradation portion of Mueller and Fusenig's proposed cycle (Mueller and Fusenig 2004; Sato et al. 2004).

Several MMPs contribute to ECM degradation in normal myometrium and uterine leiomyoma. MMP-1, large amounts of MMP-2, MMP-3 and MMP-9 are all produced and secreted by normal myometrium and leiomyoma SMC (Wolanska et al. 2004). Additionally, all of these enzymes are found in significantly greater amounts in UL tissue than healthy myometrium. MMP-2 and MMP-9, also known as gelatinases, are markedly increased in fibroids (Wolanska et al. 2004; Bogusiewicz et al. 2007). Gelatinases degrade collagen and fibronectin, which leads to the breakdown of essential components of the ECM scaffold formed with other ECM proteins. Mueller and Fusenig proposed that a constant degradation of extracellular matrix by MMPs, and the signaling induced by growth factors released in the process, leads to invasion and growth of tumors. The following section proposes how MMPs and growth factors in ULs contribute to this model, expanding the model to uterine leiomyoma progression.

As previously mentioned, MMP-2 and MMP-9 expression is significantly higher in fibroids compared to other tissues and to healthy myometrium (Bogusiewicz et al. 2007). MMP-2 is shown to degrade the surrounding ECM by binding and cleaving collagen type 1, as well as collagen type IV (Karagiannis and Pope 2006; Steffensen et al. 1995). A recent study showed that MMP-2 expression in leiomyoma is twice that of myometrium, and that MMP-2 activity in ULs showed a significant reduction in ECM deposition (Bogusiewicz et al. 2007). The increased degradation and remodeling of ECM through MMP-2 activity leads to the release of growth factors bound to ECM components.

It is well established that growth factors play an essential role in the growth and maintenance of individual cells and tissues throughout the body (Goustin et al. 1986). Several growth factors are upregulated in uterine leiomyoma compared to normal myometrium. Moore et al. studied the secretion of growth factor proteins in the ECM of cultured uterine leiomyoma tissue and found that insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), transforming growth factor beta (TGF- β), epidermal growth factor (EGF), and fibroblast growth factor (FGF) secretions are significantly increased (Moore et al. 2010).

Evidence is accumulating that suggests particular glycosaminoglycans can act indirectly on uterine SMC by binding and storing specific growth factors (Taipale and Keski-Oja 1997; Kobayashi et al. 1996). Several of the growth factors identified by Moore et al. covalently bind to extracellular matrix components (Taipale and Keski-Oja 1997). These

growth factors can be released at the appropriate time to bind to surface receptors and activate signal transduction pathways within the cell. Wolanska et al. investigated the previously identified glycosaminoglycans in uterine leiomyoma (heparan sulfate, keratan sulfate, chondroitin sulfates and heparin), their associated growth factors, and the actions of those growth factors on uterine leiomyoma growth (Wolanska et al. 2004).

Release of TGF- β from ECM and the action of TGF- β on fibroids Transforming growth factor beta (TGF- β) is a protein identified as playing both an inhibitory and stimulatory role in cell cycle progression, proliferation and hypertrophy in various tissues (Shalitin et al. 2003; Han et al. 2014; Gaarenstroom 2014). TGF- β is a family of growth factors that are synthesized and secreted by cells. TGF- β can either bind directly to receptor tyrosine kinases on neighboring cells, or TGF- β can bind to components of the ECM for temporary storage (Barnard et al. 1990). Active TGF- β binds decorin and inactive TGF- β binds latent binding proteins (Yamaguchi et al. 1990; Taipale and Keski-Oja 1997). Decorin is a single-stranded proteoglycan that binds collagen type I as well as latent binding proteins. Latent binding proteins are proteins that bind both TGF- β and fibronectin and elastin; they are thought to act as a storage reservoir for TGF- β (Taipale and Keski-Oja 1997; Svensson et al. 1995). When matrix metalloproteinases, specifically MMP-2, degrade collagen type I and other ECM components, TGF- β can be released from decorin and the latent binding proteins. TGF- β is then free to bind to TGF- β receptors on neighboring cells.

Two isoforms of the transforming growth factor beta family have been identified as growth factors involved in UL growth, TGF- β 1 and TGF- β 3 (Lee et al. 2001). Both isoforms are shown to increase cell proliferation and ECM deposition in UL compared to myometrium exposed to the same levels of TGF- β (Shalitin et al. 2003; Lee et al. 2001). Levels of TGF- β are also abnormally high in fibroids. TGF- β 3 is significantly increased in UL tissue compared to healthy myometrium (Moore et al. 2010; Lee et al. 2001).

Strong evidence exists indicating that TGF- β binding to its receptor initiates signaling through several intracellular pathways (Lee et al. 2001; Levens et al. 2005). For example, phosphorylated Smad was significantly increased in fibroids when co-cultured with fibroblasts and exposed to TGF- β compared to myometrium in the same environment (Moore et al. 2010). TGF- β 3 has been shown to upregulate phosphorylated Smad2/3 and Akt. Blocking the Smad and PI3K/Akt, mTOR pathways led to a significant decrease in the expression of collagen and fibronectin and deposition of ECM (Salama et al. 2012). Integrin-ECM signaling through MAPK/Erk is known to increase the expression specific extracellular matrix proteins and promote cell proliferation, and TGF- β 3 also increases phosphorylation of MAPK/Erk in ULs (Lee et al. 2001; Levens et al. 2005).

TGF- β has been posited as a growth factor likely to play a significant role in the increased expression and deposition of ECM in fibroids. Joseph et al. showed that treating healthy uterine SMC with TGF- β induces a change in the cells and tissue that resembles the fibrotic characteristics of ULs (Joseph et al. 2010). TGF- β is also known to increase the expression of collagen type 1 and the expression of an enzyme responsible for post-translational modification of collagen type 1, procollagen C-proteinase, in cardiac tissue fibrosis (Shalitin et al. 2003). A study in dermal fibroblasts also showed that treatment with TGF- β led to an increase in type I and type III collagen and fibronectin (Varga et al. 1987). In fibroids, TGF- β was shown to significantly increase the expression of fibromodulin, collagen type I, fibronectin and plasminogen activator inhibitor-1 compared to healthy myometrial SMC; however the downstream effectors have yet to be studied in detail (Ding et al. 2004; Levens et al. 2005).

Levens et al. also showed that treatment with TGF- β 1 had inhibitory effects on DNA synthesis in myometrial cells, but showed no effect on the DNA synthesis and proliferation of leiomyoma SMC. The same study showed that TGF- β 3 had a similar inhibitory effect on myometrial cell DNA synthesis; however, the level of DNA synthesis in UL SMCs increased significantly (Levens et al. 2005). Treating uterine leiomyoma SMC with TGF- β inhibitors significantly decreased cell proliferation and cell cycle progression, suggesting that TGF- β signaling in fibroid SMC and fibroblasts leads to both increased ECM deposition and proliferation (Salama et al. 2012; Moore et al. 2007).

Release of FGF from the ECM and the action of FGF on fibroids Fibroblast growth factor (FGF) is a protein sequestered in ECM and released upon degradation of the GAG components of the matrix. FGF is a family of growth factors involved in a wide range of signaling and biological functions, including embryonic development, neovascularization, cell proliferation, and tumorigenesis (Turner and Grose 2010).

FGF binds to the carbohydrate portion of a large proteoglycan, heparan sulfate proteoglycan (HSPG), which is an ECM protein contributing to the large volume of matrix seen in fibroids (Casimiro et al. 2014; Wolanska et al. 1998). FGF also binds and signals through fibroblast growth factor receptor (FGFR), which is found on many cell types. HSPG plays an integral role in binding FGF to its receptor, possibly via stabilizing the ligand-receptor complex and thus increasing activation of intracellular signal cascades (Wolanska and Bankowski 2006). UL tissue has a significantly elevated expression of FGFR compared to myometrium, suggesting an enhanced FGF signaling in leiomyomas (Anania; 1997; Cirilo et al. 2013).

FGF-1 and FGF-2 are the predominant FGFs active in myometrium and fibroids (Anania 1997; Moore et al. 2010). FGF-1 protein levels were found to correlate with fibroid size,

and was significantly elevated—even in small fibroids—compared to normal myometrium (Wolanska and Bankowski 2006). FGF-2 levels in both myometrium and leiomyoma were shown to be greater than FGF-1; FGF-2 was also significantly greater in UL tissue compared to myometrium (Wolanska and Bankowski 2006), suggesting that this isoform may be more important than FGF-1 in uterine pathophysiology.

FGF binding to FGFR leads to the activation of MAPK (Forsten-Williams et al. 2005; Wolanska and Bankowski 2006). FGF-2 activation of MAPK has been shown to increase expression of collagen type I and cell proliferation in fibrotic tissues (Yu et al. 2003). Moore et al. observed that the increased expression of FGF-2 in ULs correlates with increased cell proliferation rates (Moore et al. 2010). Another study showed fibroids are more sensitive to FGF signaling, and that FGF exposure leads to a significantly greater increase in DNA synthesis, than normal myometrium (Rauk et al. 1994). Although FGFs are known to increase collagen type I in other fibrotic tissues, further study is required to identify which, if any, ECM components are regulated by FGF in UL.

Release of IGF from ECM and the action of IGF on fibroids Insulin-like growth factor (IGF) is an essential protein required for growth and development during childhood, and assists in regulating growth in adult tissues (Laron 2001). IGF is also identified as a promoter of tumorigenesis in different tissues, making it a potential target for investigation in fibroids (Moschos and Mantzoros 2002).

Most IGF is produced and secreted by the liver and is transported to other tissues. It is transported through the bloodstream via insulin-like factor binding protein (IGFBP), a family of carrier proteins that are bound to most of the IGF in the body. IGF also binds IGFBP in cells and tissues, where IGFBP is produced by tissue fibroblasts and secreted into the ECM (Laron 2001; Clemmons 1991).

Several IGFBPs bind IGF, but IGFBP-5 is shown to bind specifically to components of the ECM. IGFBP-5 binds to collagen type III and IV, laminin, and fibronectin (Jones et al. 1993). IGFBP-5 affinity for IGF-I and IGF-II is greater than that of the IGF receptor, demonstrating the great capacity for IGFBP-5-binding to modify the availability of free IGF to bind to receptors on adjacent cells (Jones et al. 1993). SMC, such as those in fibroids, secrete a protease that cleaves IGFBP-5, causing the release of IGF (Salama et al. 2012). Lysis of IGFBP-5 allows release of IGF to bind the IGF receptor tyrosine kinase on adjacent cells.

Several studies show that IGF-1 expression is significantly upregulated in ULs. One study reported a 7.5 fold increase in IGF-1 expression in fibroids when compared to age-matched myometrium (Giudice et al. 1993; Burroughs et al. 2002). A study by Yu et al. showed that ULs have a significantly increased expression of IGF and IGFR (Yu et al. 2008). They also showed that a higher fraction of IGF receptors are phosphorylated in UL

than in healthy tissue. Phosphorylation by receptor tyrosine kinases induced by IGF-1 binding also corresponds with much more rapid and increased phosphorylation of downstream effectors in fibroid SMC (Yu et al. 2008).

IGF-1 binding to IGFR also leads to downstream phosphorylation of protein kinase B, better known as AKT (Peng et al. 2009). AKT phosphorylation increases cell proliferation and cell viability in studies of hormone-activated AKT pathways in fibroids (Hoekstra et al. 2009). A study by Peng et al. examined 50 cases of UL and the effect of IGF-1 phosphorylation of AKT on cell proliferation. They observed that IGF-1 phosphorylation of AKT is strongly correlated with fibroid size, suggesting that the higher levels of IGF-1 may contribute to increased fibroid size (Peng et al. 2009). Yu et al. also found that fibroids exposed to increased levels of IGF-1 had significantly faster cell proliferation rates when compared to normal tissue (Yu et al. 2008). However, it should be noted that more work is needed to determine the cause-effect nature of the relationship between increased IGF levels and fibroid size.

IGF-1 increases cell survival by inhibiting apoptosis in many cells and tissues, including fibroids. Gao and colleagues investigated the action of IGF-1 on Bcl-2 expression, an anti-apoptotic protein. They found that IGF-1 treatment led to increased Bcl-2 protein levels in fibroids, indicating a likely apoptotic pathway in which IGF-1 could increase cell survival (Gao et al. 2001).

IGF-1 is also shown to increase collagen production by amplifying the effects of growth hormone in human dermal tissue (Edmonson et al. 2003). Studies of IGF-1 regulation of collagen production in UL have yet to be conducted; however, evidence suggests that TGF- β is not the only growth factor increasing ECM deposition in fibroids, as noted earlier (Moore et al. 2010). Further investigation of the effects of IGF on ECM protein expression may provide important insights and may reveal potential therapeutic targets.

A recent study showed the IGF-2 isoform may be contributing to UL growth. IGF-2 is known to play a role in malignant tumors as a biomarker indicating the prognosis of many cancers (Yamamoto et al. 2013). Recent studies showed that IGF-2 expression is significantly increased in fibroids (Csatlós et al. 2013; Di Tommaso et al. 2013). A study comparing a large sample of women with and without fibroids showed that IGF-2 polymorphisms are associated with an increased susceptibility to fibroids, supporting the findings that IGF-2 may play a more important role in UL incidence and progression than previously thought (Yu et al. 2008). However, evidence suggests that IGF-2 does not signal through the same pathways as IGF-1, thus requiring further investigation into the actions of IGF-2 on fibroids (Peng et al. 2009).

ECM release of PDGF and the action of PDGF on fibroids Platelet-derived growth factor (PDGF) is a protein that is important for the motility and cell cycle progression in

many tissues (Hannink and Donoghue 1989). PDGF binds to a binding site on extracellular fibrinogen and fibronectin (Martino et al. 2013; Lin et al. 2011). Lin et al. also found that cell survival is dependent on PDGF binding to fibronectin in the extracellular matrix, as fibronectin-null cells were unable to survive even in the presence of PDGF (Lin et al. 2011). A recent study showed fibronectin modulates PDGF binding to the PDGF receptor on nearby cells (Martino et al. 2011).

Several isoforms of PDGF have been identified in human tissues. Moore et al. found that PDGF expression is significantly increased in fibroids compared to myometrium (Moore et al. 2010). A study by Suo et al. compared mRNA levels of UL and myometrial tissues. The study found that the amounts PDGF-A and PDGF-B isoforms were similar in postmenopausal myometrium and leiomyoma; however, PDGF-C was significantly higher in UL compared to myometrium (Suo et al. 2009). PDGF-C has not been examined in premenopausal women, but PDGF-A and PDGF-B were significantly increased in the fibroids of premenopausal women compared to the myometrium (Liang M1 et al. 2006).

PDGF is shown to stimulate proliferation of arterial SMC (Sejersen et al. 1986). More recent studies show PDGF promotes cell proliferation and accelerates cell cycle progression in fibroid SMC when compared to normal myometrium (Koohestani et al. 2013). Not only were protein levels higher in UL, but evidence suggests that PDGF signaling is greater as well. PDGF receptor expression is also significantly greater on the surface of fibroid SMC (Fayed et al. 1989).

PDGF binding to its receptor in fibroid SMC results in phosphorylation of MAPK, similar to TGF- β and FGF. Treating fibroid SMC with PDGF leads to a significant increase in phosphorylated MAPK/Erk and a significant increase in the number of cells in S phase (Koohestani et al. 2013). Although PDGF does not directly increase ECM deposition in fibroids on a per cell basis, the overall increase in cell number leads to an increased total expression of ECM components, thus contributing to the growth and size of ULs.

Release of EGF from the ECM and the action of EGF on fibroids Epidermal growth factor (EGF) is a family of proteins that are produced and secreted by numerous tissues in the body. They are strongly expressed in wound healing and cancer (Zeng et al. *In press*). EGF is present in either the unbound form or bound to an EGF binding site on heparin, a highly sulfated GAG that also binds FGF. Heparin-bound EGF is capable of binding to the EGF receptor, a receptor tyrosine kinase that dimerizes upon EGF binding, and begins a well-characterized intracellular signaling cascade (Block et al. 2004).

As is the case with the other growth factors already discussed, EGF is overexpressed in UL tissue when compared to healthy myometrium (Moore et al. 2010). EGF is shown to increase cell proliferation, adhesion, and fibroblast migration in many cells and tissues (You and Nam 2013). EGF is shown

to induce cell proliferation and DNA synthesis in fibroids; however, the phosphorylation of downstream MAPK is sustained for a greater period of time in normal myometrial SMC (Lin et al. 2011). Wang et al. found similar results when comparing EGF receptor activation with heparin-bound EGF on fibroids and myometrium; the latter demonstrated higher levels of MAPK phosphorylation than the former (Wang et al. 2005).

These findings suggest that EGF may have a more significant role in the myometrium than in the pathogenesis of fibroids. However, EGF is the least well-studied of the major growth factors in uterine pathophysiology, and much more work is needed before the functions of EGF in that organ is known.

Non-surgical/Non-invasive therapeutic targets for ECM reduction in fibroids

Current treatment opportunities for uterine leiomyoma are primarily surgical: hysterectomy, myomectomy, resection, thermal ablation, or uterine artery embolization (UAE) (Khan et al. 2014). With the exception of hysterectomy, all of these treatment options have significant recurrence rates and/or fertility problems. A recent study of UAE noted a significant reduction in uterine fibroid size and symptoms; however, of the cohort of women studied, only one was able to conceive post-UAE, and that patient eventually miscarried (Torre A1 et al. 2014). Another study found that younger women undergoing UAE had a higher chance of UL recurrence than older women closer to menopause, suggesting UAE may not be effective in preventing recurrence of fibroids in women of reproductive age (Scheurig-Muenkler C1 et al. 2013). Although the cause of post-UAE subfertility is not known, it is possible that the substantial inflammatory response that accompanies the necrosis is the culprit.

Laparoscopic myomectomy has been shown to be an effective method of treating UL when a single fibroid is thought to be the problem, and the recurrence rate for this treatment is relatively low; however, women undergoing myomectomy also exhibit reduced fertility, again most likely due to the inflammation that results from the procedure. Clearly, less invasive therapies that preserve fertility are desirable in treating this condition (Tinelli A1 et al. 2012).

Evidence of ECM deposition in uterine leiomyoma stimulated by various hormones and growth factors and working through intracellular signaling pathways has created the potential to develop treatment regimens that may reduce the size and symptoms of uterine leiomyoma. There are currently several new therapies targeting ECM reduction, including opportunities to directly digest the current excess ECM, to inhibit upstream propagation of ECM deposition, and also indirectly by reducing cell proliferation. Unfortunately, none

of these has shown the promise in clinical trials that they displayed in pre-clinical animal studies, but there is certainly reason for optimism that non-surgical therapies that preserve fertility may come to fruition in the next several years.

Hormone and hormone receptor agents The ECM in fibroids could potentially be reduced through the use of receptor antagonists or very weak agonists for the hormones thought to be responsible for UL growth, namely estrogen and progesterone. Asoprisnil is a weak progesterone agonist that reduced the symptoms associated with fibroids early on in clinical trials (Chwalisz et al. 2007). The study also showed a reduction in uterine volume, and Morikawa et al. investigated the action of asoprisnil on uterine leiomyoma. Their study confirmed past findings that asoprisnil significantly decreases cell proliferation and collagen type I and III expression in ULs compared to myometrium also treated with asoprisnil (Morikawa et al. 2008). Asoprisnil is also shown to decrease the expression of growth factors involved in ECM deposition in UL, including IGF and TGF- β 3 (Wang et al. 2006). However, asoprisnil did not fare well in Phase III clinical trials because of modest efficacy and significant side effects and is not approved for clinical use.

Gonadotropin-releasing hormone (GnRH) is shown to have an indirect effect in increasing uterine leiomyoma size by increasing the secretion of progesterone (Takeuchi et al. 2000). The Food and Drug Administration has approved one agonist of GnRH, leuprolide acetate, also known as Lupron, as a presurgical treatment for uterine leiomyoma. Leuprolide acetate decreases cell proliferation and downstream hormone production in UL (Watanabe et al. 1992). Britten et al. chose to investigate the efficacy of cetrorelix acetate, another GnRH agonist, compared to leuprolide acetate on reducing uterine leiomyoma extracellular matrix components (Britten et al. 2012). The study found cetrorelix acetate had a statistically significant reduction in collagen, fibronectin and versican expression compared to untreated leiomyoma and leiomyoma treated with leuprolide acetate (Britten et al. 2012).

Use of GnRH and progesterone agonists may be effective in reducing ECM components in UL; however, the side effects of bone density loss, increased bleeding, and hot flashes may outweigh the benefits of these therapies (Khan et al. 2014). In addition, these treatments are not viable options for women who wish to get pregnant, and they are generally more appropriate for tumor reduction prior to surgery.

Naturally occurring and plant-derived compounds Naturally occurring compounds in humans, plants and foods have been identified as potentially effective prevention and treatment opportunities for many cancers (Ravindran et al. 2009; Moran et al. 2013). Curcumin, a natural phenol found in the spices turmeric and cumin, is shown to have anti-proliferative properties in various cancers, and a recent study investigated

the efficacy of curcumin on decreasing ECM in fibroids (Ravindran et al. 2009; Malik M1 et al. 2009). The study found a dose-dependent reduction in UL smooth muscle cell proliferation when compared to myometrium SMC with concentrations of curcumin 10–15 μM . A further increase in curcumin concentration adversely reduced myometrium proliferation, showing that lower concentrations of curcumin are sufficient to significantly inhibit UL SMCs proliferation while permitting normal SMC growth. Similar concentrations are shown to induce apoptosis in UL SMCs, determined by the increased expression of caspase-3 and caspase-9. Erk1/2 was also significantly reduced, and fibronectin gene expression was significantly reduced upon curcumin treatment (Malik M1 et al. 2009).

A similar approach was used in looking at the retinoic acid pathway in treating ULs. Vitamin A, or retinol, is metabolized to all-trans retinoic acid via alcohol dehydrogenase and aldehyde dehydrogenase. Retinoic acid goes on to bind retinoic acid receptors in specific tissues throughout the body and is thought to decrease and prevent certain cancers (Shapiro SS1 et al. 2013). Gilden et al. used liarozole, a retinoic acid metabolic blocking agent that blocks CYP450-mediated catabolism of retinoic acid. Liarozole treatment significantly decreased cell proliferation in both leiomyoma and myometrium tissues, and altered ECM gene expression. The study showed no statistically significant change in ECM components upon treating myometrium, except for fibromodulin, but showed a statistically significant reduction in collagen type I, collagen type II, fibromodulin, fibronectin and total versican in uterine leiomyoma (Gilden et al. 2012).

Othman and colleagues compared polymorphisms in the retinoic acid receptor, among other receptors, between African American women and Caucasian women in the United States (Othman 2008). The study found that the expression of retinoic acid receptors in African American women was significantly lower than that of Caucasian women, concluding that there may be decreased action of retinoic acid on target tissues in African American women (Othman 2008). This finding generally supports the idea that retinoic acid may reduce fibroids, but further studies are needed to confirm the efficacy of retinoic acid *in vivo*, and to determine whether there is a gender-specific effect.

A large cohort study randomly enrolling premenopausal black and white women, screened participants using ultrasound and then assessed Vitamin D storage. The study controlled for potential confounding variables and found women who had adequate levels of vitamin D were at 32 % lower odds of having uterine leiomyoma compared to women with vitamin D insufficiency (Baird et al. 2013). A recent case control study by Paffoni and associates found that women with a vitamin D deficiency were 2.4 times more likely to have a diagnosis of fibroids than women with a adequate levels of vitamin D (Paffoni and Somigliana 2013). Other recent studies showed

that 1,25-dihydroxyvitamin D3 significantly shrank UL tumor size and inhibited human leiomyoma SMC proliferation in rat models (Halder et al. 2012; Sharan et al. 2011).

Halder et al. also investigated the action of 1,25-dihydroxyvitamin D3 on uterine leiomyoma by comparing gene expression of ECM components with untreated tissue. The study focused primarily on TGF- β 3 action on UL ECM deposition by investigating the pathways TGF- β 3 signals in uterine leiomyoma. The study found that, upon treatment with Vitamin D3, both the Smad pathway and Erk1/2 pathway were downregulated. Phosphorylated Smad2, Smad3, Smad4, Erk were all downregulated in a dose-dependent response to Vitamin D3 treatment. Equally important, the study showed that Vitamin D3 treatment of human leiomyoma SMC resulted in a statistically significant decrease in expression of collagen type I and fibronectin, suggesting that Vitamin D3 could reduce TGF- β 3 induced fibrosis in fibroids (Halder et al. 2011).

TGF- β 3 was also the therapeutic target in another study, using 2-methoxyestradiol, a naturally occurring estradiol metabolite that has low affinity for the estrogen receptor (Salama et al. 2012). The same lab had previously shown that 2-methoxyestradiol inhibited cell proliferation, promoted apoptosis, and reduced collagen production in both rat and human fibroid SMC (Salama et al. 2006). Salama et al. later investigated the inhibited TGF- β 3 pathway by 2-methoxyestradiol. The study found that 2-methoxyestradiol inhibited both the TGF- β -dependent Smad and PI3k/Akt/mTOR pathways, leading to the decreased the expression of collagen type I and type III (Salama et al. 2012). This compound remains potentially useful as a fibroid therapy and is currently under intensive investigation.

Other natural compounds have been tested that reduce IGF-1 in uterine leiomyoma. *Scutellaria barbata* D. Don (SB) is a perennial herb grown in China and Korea, and SB was used to reduce IGF-1 in uterine leiomyoma. SB treatment of uterine leiomyoma reduced cell proliferation in UL cells and also reduced IGF-1 expression significantly to levels similar to normal myometrium (Kim et al. 2005). The reduction of IGF-1 to levels of normal myometrium with SB treatment, without significantly reducing IGF-1 levels in myometrium, provides a promising therapy for uterine leiomyoma. Further research in these therapies is necessary to investigate the reduction in ECM deposition.

While the potential of nutritional interventions or the use of naturally occurring compounds is very appealing, the evidence that any nutritional supplement is highly effective against human fibroids, combined with the narrow dose response and the difficulty in providing such supplements in accurate and consistent dosages, makes it unlikely that curcumin or other similar compounds will be useful in individual patients, with the possible exception of green tea derivatives. The epigallocatechin gallate components of green tea have been the subject of significant research as well as clinical trials for

several cancers. Their effectiveness is supported by epidemiological studies as well. Recently, Al-Hendy and colleagues obtained promising results using green tea extracts in a murine model for fibroids (Zhang et al. 2010).

Other therapies targeting reducing growth factor-mediated mechanisms of ECM deposition This review discussed earlier that ECM deposition can increase as a direct or indirect result of growth factor expression in uterine leiomyoma. IGF and TGF- β not only increased cell proliferation and cell survival in uterine leiomyoma, but also their downstream signaling leads to increased expression of ECM components. Therapies in UL and other fibrotic tissues seek to inhibit IGF and TGF- β signaling. The current avenues of investigation of TGF- β investigation are focused on plant, food, or naturally occurring compounds and have been discussed above.

Recent studies have suggested ways to regulate IGF action on SMC that would reduce downstream cell proliferation and ECM deposition. One study used a protease-resistant IGFBP-5 construct to bind IGF-1 in the extracellular matrix, avoiding the eventual degradation of IGFBP-5 and release of IGF. PI3K phosphorylation was reduced downstream upon treating the SMC with protease-resistant IGFBP-5 (Imai Y1 et al. 1997). Xu et al. used an anti-fertility treatment, levonorgestrel, to target IGF expression in fibroid SMC. IGF expression was significantly reduced with small concentrations of levonorgestrel when compared to untreated controls. These investigators also found that levonorgestrel decreased fibroid SMC proliferation (Xu et al. 2010). Unfortunately, further evidence has not been forthcoming on this compound.

MMPs as potential therapeutic targets As previously mentioned, MMP-9 and especially MMP-2 are significantly increased in UL, and their enzymatic activity promotes tumor growth and ECM deposition. Relaxin is a peptide hormone expressed by the ovary, placenta and other reproductive tissues in both men and women (MacLennan 1991). Relaxin is also thought to reduce myofibroblast proliferation, ECM deposition and MMP expression in fibrotic tissues (Kang et al. 2014). These investigators showed that treatment of cultured myofibroblastic cells with relaxin gene therapy reduced MMP-2 by 70 %, MMP-9 by 80 %, fibronectin by 74 % and collagen types I and III by 22 % and 48 % respectively (Kang et al. 2014). This and related gene therapy approaches represent potentially exciting approaches that warrant further investigation.

Conclusion

The extracellular matrix components and interactions with uterine leiomyoma smooth muscle cells create a range of

diverse signaling pathways within a complex tissue milieu. Fibroids afflict tens of millions of American women, and the current research and treatment opportunities are crude and ineffective at preserving fertility when compared to treatment for other tumors or any disease with a high incidence in the population.

Collagen, fibronectin and sulfated proteoglycans are over-expressed in UL compared to normal myometrium and contribute to the volume of ECM deposition and the size of fibroids. Integrins expressed on SMC interact with ECM components in fibroids, leading to the initiation of several intracellular signaling cascades that result in both cell proliferation and ECM production. Integrin β 1 is significantly over-expressed in UL and integrin β 1 interactions with fibroid ECM components results in increased cell proliferation and ECM deposition. Sulfated proteoglycans act as co-receptors for some growth factors, and provide storage reservoirs for others.

Several growth factors appear to regulate UL growth and progression, including TGF- β , IGF, PDGF, EGF and FGF. TGF- β and IGF are thought to directly increase the amount of ECM. PDGF, EGF and FGF increase cell proliferation and cell cycle progression, thus indirectly contributing to the increased volume of fibroids by providing more cells that are producing ECM.

Despite the currently limited treatment opportunities for fibroids, there is great potential for improvement. Understanding the chromosomal and genetic abnormalities that appear to be critical in more than a third of fibroids—a topic not within the purview of this review—represents one such avenue. What is desperately needed to make progress in developing non-surgical therapies that preserve fertility is a sound understanding of the basic physiological and molecular mechanisms regulating the pathogenesis of these tumors. There is a dearth of both investigators, and most importantly funding, committed to this area. For example, epidemiologists could undoubtedly unravel the genetic and environmental basis for the large discrepancy in fibroid incidence, number, and severity between black women and other women if sufficient funds were made available to examine this issue. Cytogeneticists could readily determine which of the many chromosomal abnormalities present in fibroids are causal and could ferret out the specific genes that are involved—especially since each fibroid is clonal in nature, i.e., it arises from a single cell in the myometrium—if adequate resources were made available. Cell and molecular biologists currently have all the tools necessary to understand the underlying mechanisms involved in uterine SMC proliferation and ECM deposition, but simply lack the necessary funding to do so. The pathogenesis and treatment of fibroids, the most common tumor in women, is hindered most by the lack of resources available and not by some mysterious or complex pathophysiology beyond our ken.

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