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Extracellular matrix in uterine leiomyoma pathogenesis: a potential target for future therapeutics

Md Soriful Islam[1,2], Andrea Ciavattini[3], Felice Petraglia[4], Mario Castellucci[1], and Pasquapina Ciarmela[1,5,*]

[1]Department of Experimental and Clinical Medicine, Faculty of Medicine, Università Politecnica delle Marche, via Tronto 10/a, Ancona 60020, Italy [2]Biotechnology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh [3]Department of Clinical Science, Università Politecnica delle Marche, Ancona, Italy [4]Department of Experimental and Clinical Biomedical Sciences 'Mario Serio', Obstetrics and Gynecology, University of Florence, Italy [5]Department of Information Engineering, Università Politecnica delle Marche, Ancona 60131, Italy

*Correspondence address: Department of Experimental and Clinical Medicine, Faculty of Medicine, Università Politecnica delle Marche, Ancona, Italy. Tel:+39-0712206270, Fax:+39-0712206087, E-mail: p.ciarmela@univpm.it orcid.org/0000-0002-4400-3786

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BACKGROUND: Uterine leiomyoma (also known as fibroid or myoma) is the most common benign tumor of the uterus found in women of reproductive age. It is not usually fatal but can produce serious clinical symptoms, including excessive uterine bleeding, pelvic pain or pressure, infertility and pregnancy complications. Due to lack of effective medical treatments surgery has been a definitive choice for the management of this tumor.

OBJECTIVE AND RATIONALE: Extracellular matrix (ECM) accumulation and remodeling are thought to be crucial for fibrotic diseases such as uterine leiomyoma. Indeed, ECM plays important role in forming the bulk structure of leiomyoma, and the ECM-rich rigid structure within these tumors is thought to be a cause of abnormal bleeding and pelvic pain. Therefore, a better understanding of ECM accumulation and remodeling is critical for developing new therapeutics for uterine leiomyoma.

SEARCH METHODS: PubMed and Google Scholar were searched for all original and review articles/book chapters related to ECM and medical treatments of uterine leiomyoma published in English until May 2017.

OUTCOMES: This review discusses the involvement of ECM in leiomyoma pathogenesis as well as current and future medical treatments that target ECM directly or indirectly. Uterine leiomyoma is characterized by elevated levels of collagens, fibronectin, laminins and proteoglycans. They can induce the mechanotransduction process, such as activation of the integrin-Rho/p38 MAPK/ERK pathway, resulting in cellular responses that are involved in pathogenesis and altered bidirectional signaling between leiomyoma cells and the ECM. ECM accumulation is affected by growth factors (TGF-β, activin-A and PDGF), cytokines (TNF-α), steroid hormones (estrogen and progesterone) and microRNAs (miR-29 family, miR-200c and miR-93/106b). Among these, TGF-βs (1 and 3) and activin-A have been suggested as key players in the accumulation of excessive ECM (fibrosis) in leiomyoma. The presence of elevated levels of ECM and myofibroblasts in leiomyoma supports the fibrotic character of these tumors. Interestingly, ECM may serve as a reservoir of profibrotic growth factors and enhance their activity by increasing their stability and extending their duration of signaling. At present, several classes of compounds, including gonadotropin-releasing hormone (GnRH) agonist (leuprolide acetate), GnRH antagonist (cetrorelix acetate), selective progesterone receptor modulators (ulipristate acetate and asoprisnil), antiprogestin (mifepristone) and natural compounds like vitamin D and resveratrol have been studied as medical treatments that target ECM in uterine leiomyoma.

WIDER IMPLICATIONS: Although several types of drugs (mostly antiproliferative agents) are available for leiomyoma treatment, none of them were introduced specifically as antifibrotic agents. In light of its critical role in the process of fibrosis in leiomyoma, we propose that ECM should be considered as a crucial target for future therapeutics. Thus, the introduction of drugs that are specifically antifibrotic could be a good solution to control abnormal leiomyoma growth and associated clinical symptoms. The antifibrotic compounds can be introduced based on their ability to regulate ECM components and their receptors, as well as growth factors, cytokines, steroid hormones and their corresponding receptors and intracellular signaling pathways, as well as microRNAs, involved in ECM production in leiomyoma.

Key words: uterine leiomyoma / fibrosis / extracellular matrix / growth factors / steroid hormones / mechanotransduction / inflammation / myofibroblast / medical treatment / ultrastructure

Introduction

Uterine leiomyoma (also known as fibroid or myoma), the most common benign tumor of the uterus, is found in women of reproductive age (Islam *et al.*, 2013a). They may occur in more than 70% of women, and ~25% of women show clinically significant lesions (Buttram and Reiter, 1981; Cramer and Patel, 1990). Particularly, African-American women are reported to have a higher leiomyoma incidence (Marshall *et al.*, 1997; Day Baird *et al.*, 2003), with more severe clinical symptoms (Kjerulff *et al.*, 1996) compared to Caucasians. Symptoms associated with leiomyoma include excessive uterine bleeding, pelvic pain or pressure, infertility and pregnancy complications (Buttram and Reiter, 1981). Due to lack of effective, non-invasive medical treatments (Islam *et al.*, 2013b), surgery has been the primary choice for leiomyoma management. However, surgery is risky and expensive and negatively affects the quality of life of patients. In the USA, the annual economic burden of uterine leiomyoma is estimated to be between $5.9 and $34.4 billion (Cardozo *et al.*, 2012).

The precise molecular and cellular changes that led to the development and growth of uterine leiomyoma are not well understood. However, one distinguishing characteristic of uterine leiomyoma is the excessive accumulation of extracellular matrix (ECM) components including collagens (Fig. 1), fibronectin, laminins and proteoglycans (Stewart *et al.*, 1994; Arici and Sozen, 2000; Norian *et al.*, 2009; Malik *et al.*, 2012; Herndon *et al.*, 2016). High levels of ECM proteins in uterine leiomyoma result in mechanotransduction, a process whereby increased tissue stiffness leads to bidirectional signaling via integrins and downstream mediators including Rho/p38 MAPK/ERK (Rogers *et al.*, 2008; Malik *et al.*, 2012; Chen *et al.*, 2013; Thorne *et al.*, 2015) (Fig. 2). Uterine leiomyoma also expresses proteolytic enzymes such as matrix metalloproteinases (MMPs) and tissue

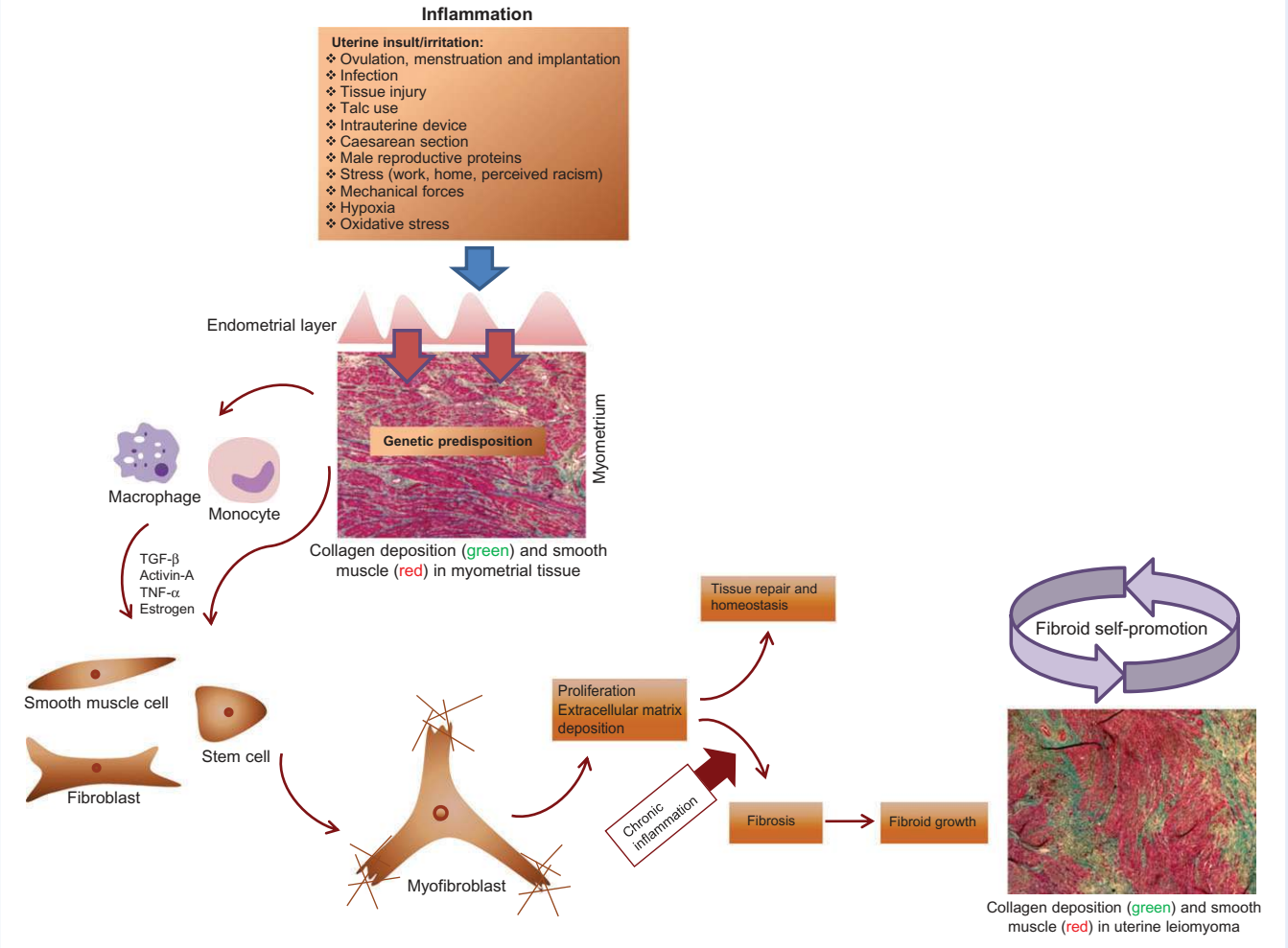

Figure 1 Hypothetical presentation of fibrosis in uterine leiomyoma. Fibrosis is initiated by inflammation in the presence or absence of genetic changes. Growth factors, cytokines and steroid hormones are produced at the site of injury and contribute to fibroblast activation and differentiation into myofibroblasts. Myofibroblasts produce ECM components to restore homeostasis and then should be eliminated by apoptosis. During chronic inflammation, myofibroblasts become resistant to elimination by apoptosis and produce excessive amounts of ECM components, leading to fibrotic transformation. Once established, fibroids can promote their own growth. Masson's trichrome stain highlighting collagen (green) and smooth muscle (red) in myometrium and leiomyoma was performed in our laboratory.

inhibitors of MMPs (TIMPs) that play key roles in ECM remodeling (Bogusiewicz *et al.*, 2007; Malik *et al.*, 2010). Furthermore, a number of studies indicate that ECM accumulation and function is regulated by growth factors (Joseph *et al.*, 2010; Islam *et al.*, 2014a), cytokines (Wang *et al.*, 2015) and steroid hormones (Qiang *et al.*, 2014). ECM binds and sequesters growth factors to promote their stability as well as restrict their activity. By degrading ECM components, MMPs and other proteolytic enzymes release growth factors and trigger activation of multiple signal transduction pathways (Fig. 3).

Uterine leiomyoma is thought to be a consequence of an improper inflammatory response (Wegienka, 2012; Leppert *et al.*, 2013) with myofibroblasts possibly playing a key role in the development of fibrosis (Feng *et al.*, 2016; Protic *et al.*, 2016) (Fig. 1). In our recent study, we found α-smooth muscle actin (α-SMA) positive and desmin negative cells as well as a large amount of collagen in leiomyoma tissue, indicating the presence of myofibroblasts and their role in the ECM deposition (Protic *et al.*, 2016). It is known that when women reach reproductive age, events such as ovulation, menstruation and implantation may create physiological injuries in the uterus. In addition to reproductive events, harmful stimuli, mechanical forces, hypoxia and oxidative stress may create a chronic inflammatory state in the uterus (Wegienka, 2012; Fletcher *et al.*, 2013; Leppert *et al.*, 2013; Santulli *et al.*, 2013). In the inflammatory state associated with injury, myofibroblasts produce ECM to promote subsequent repair processes and tissue homeostasis (Wynn, 2007). However, during chronic inflammation, myofibroblasts continuously and excessively produce ECM resulting in pathological fibrosis (Fig. 1). It is hypothesized that growth factors including TGF-βs and activin-A are key players in driving myofibroblast differentiation during the process of fibrosis (Joseph *et al.*, 2010; Islam *et al.*, 2014a; Feng *et al.*, 2016; Protic *et al.*, 2016).

ECM accumulation is a critical event in producing the rigid structure of leiomyoma, and ECM stiffness is thought to be a cause of

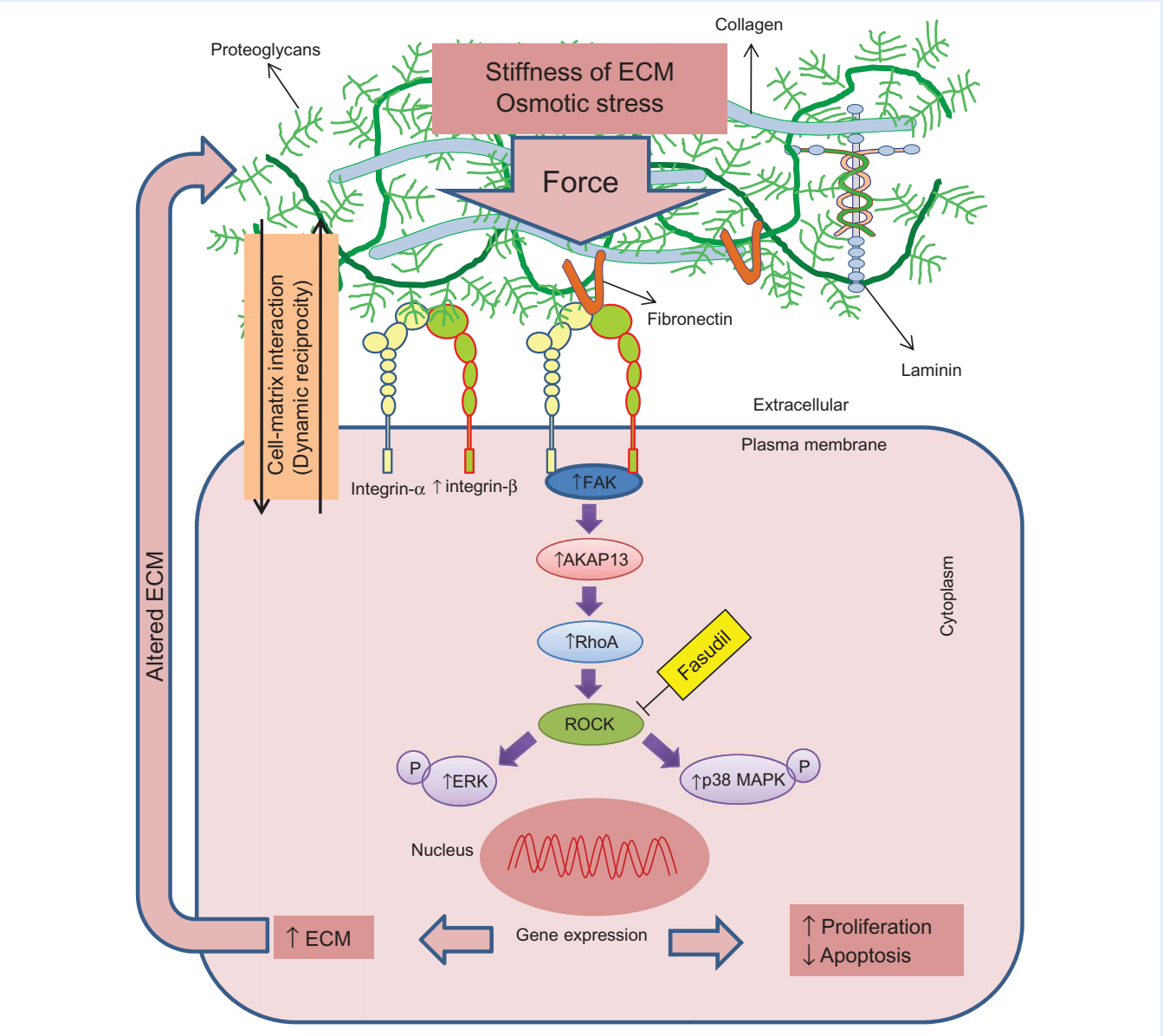

Figure 2 Mechanotransduction is caused by extracellular matrix in uterine leiomyoma cells. Mechanical force from the stiffness of ECM or osmotic stress activates mechanical signaling pathways through heterodimeric integrins (α and β) that function as transmembrane receptors for ECM components. Activation of FAK initiates actin polymerization and AKAP13 activation of RhoA, which in turn interacts with ROCK and activates the ERK/p38 MAPK-signaling cascade, resulting in changes in cell proliferation, decreased apoptosis and upregulation of genes involved in ECM composition and remodeling. Cells sense the mechanical force from newly generated altered ECM and further activate mechanical signaling that affects cell behavior and alters overall remodeling of the matrix. This process initiates cell-matrix interactions in a rapid, transient, and bidirectional manner, which is referred to as dynamic reciprocity. Compounds that can interrupt this signaling pathway are also listed (yellow square). FAK, focal adhesion kinase; AKAP13, A kinase anchor protein 13; RhoA, Ras homolog gene family, member A; ROCK, Rho kinase; ERK, extracellular signal–regulated kinase; MAPK, mitogen-activated protein kinase.

abnormal bleeding and pelvic pain or pressure. Therefore, the inhibition of further accumulation of ECM and associated fibrosis might be an option for the management of these tumors. In this review, we highlight the involvement of ECM components in leiomyoma pathogenesis. Particularly, we discuss the basis for the increase in abundance and regulation of ECM in leiomyoma as well as the role of the ECM as reservoir for growth factors and a modulator of their actions. We also present options for medical treatments that target ECM formation, which are currently used or may be attractive in the future.

Methods

We used multiple strategies to identify primary research publications and review articles/book chapters related to extracellular matrix and medical

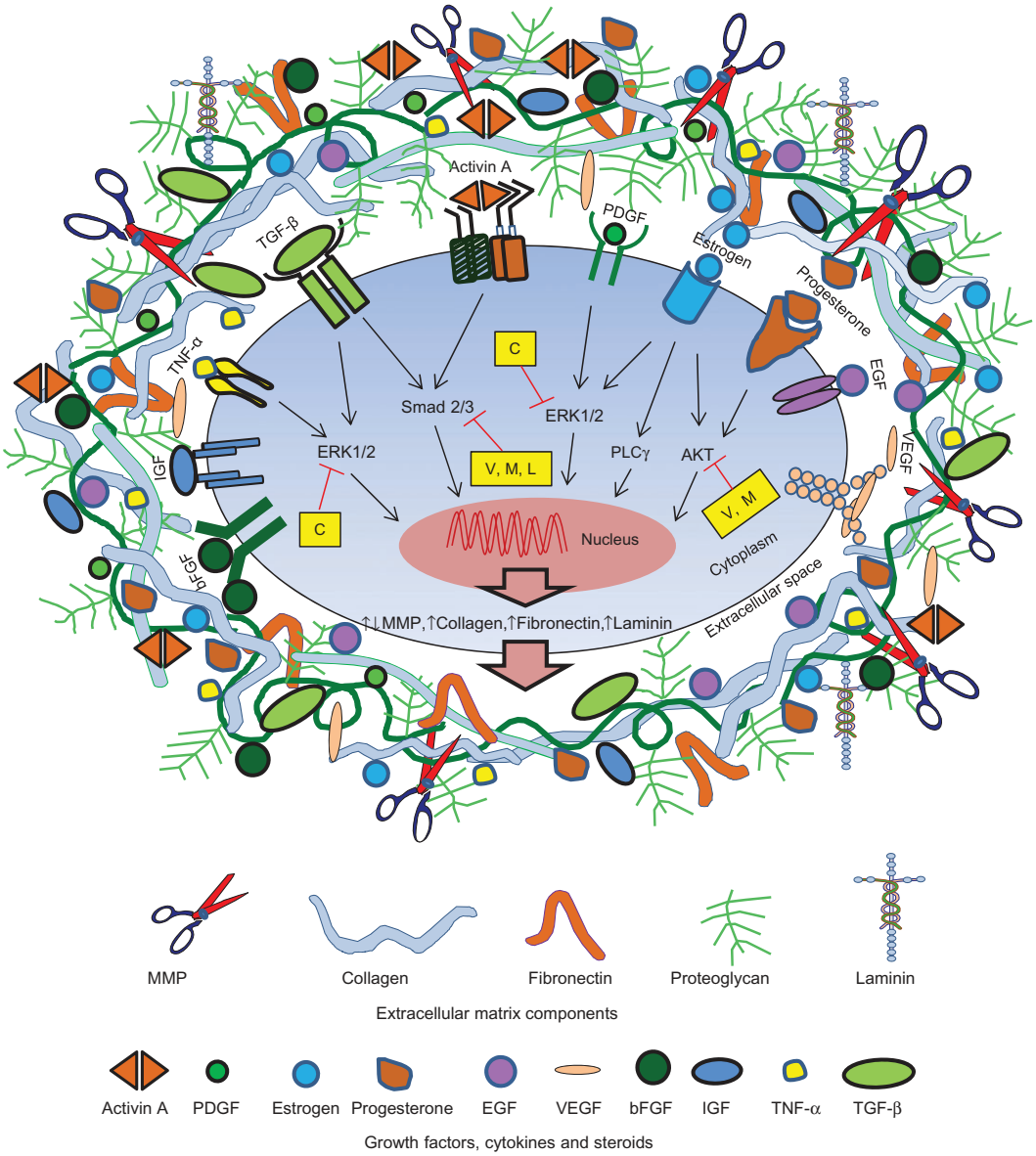

Figure 3 Regulation of extracellular matrix by growth factors, cytokines and steroid hormones in uterine leiomyoma. Extracellular matrix may act as both a reservoir of growth factors and a modulator of their actions. Compounds that can interrupt the signaling pathways are also listed (yellow squares). C, Curcumin; V, Vitamin D3; M, 2-Methoxyestradiol; L, Leuprolide acetate.

treatments of uterine leiomyoma published in English until May 2017. We conducted extensive searches in PubMed and/or Google Scholar using the following keywords either alone or in combination with 'uterine leiomyoma and extracellular matrix': sarcolemma, caveolae, myofilaments and intermediate filament, collagen, fibronectin, laminin, versican, proteoglycan, MMP, TIMP, mechanotransduction, mechanical stress, growth factor, transforming growth factor-β (TGF-β), activin-A, platelet-derived growth factor (PDGF), cytokines, chemokines, tumor necrosis factor-α, interleukin (IL)-1β, IL-11, IL-13, granulocyte-macrophage colony-stimulating factor (GM-CSF), estrogen, progesterone, microRNA, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), inflammation, myofibroblast, fibrosis, medical treatment, pharmacological treatment, leuprolide acetate, cetrorelix acetate, asoprisnil, ulipristal acetate, mifepristone, raloxifene, CP8947, 2-methoxyestradiol, liarozole, all-trans retinoic acid, vitamin D, celecoxib, tranilast, pirfenidone, halofuginone, curcumin, resveratrol and collagenase *Clostridium histolyticum*. We also searched additional relevant articles in the bibliographies of downloaded articles. Overall, we reviewed most relevant articles and included them as appropriate.

Ultrastructural features of uterine leiomyoma

A number of studies have identified some ultrastructural features that may distinguish uterine leiomyoma from myometrium. These include

sarcolemma (Richards et al., 1998), caveolae (Richards et al., 1998), extracellular matrix (Leppert et al., 2004; Wortham et al., 2006), myofilaments and intermediate filaments (Eyden et al., 1992; Wortham et al., 2006). Sarcolemma is the cell membrane of a striated muscle fiber cell and sarcolemmal dense bands were found to be significantly greater in length in fibromyomata and host myometria (non-neoplastic myometrial portion of fibromyomatous uteri) than in normal myometria (Richards et al., 1998). Caveolae are 50–100 nm invaginations of the plasma membrane with an omega (Ω) shape and the numbers of caveolae in host myometria and fibromyomata were found to be decreased in comparison to normal myometria (Richards et al., 1998). Collagens are central structural components of the ECM. In myometrium, collagen structure is ordered with tight bundles, whereas in leiomyoma, they are more loosely packed, with little common orientation (Leppert et al., 2004; Wortham et al., 2006). Myofibrils, composed of myofilaments, are rod-like units of a muscle cell. Eyden et al. reported an abundance of myofilaments with focal densities in both normal myometrium and leiomyomata with the organization of intermediate filaments being alike in both tissues (Eyden et al., 1992). However, a later study reported that myofilaments and intermediate filaments were ordered and aligned in the myometrium while disorganized structures were found in leiomyoma (Wortham et al., 2006).

Extracellular matrix in uterine leiomyoma

The hallmark of uterine leiomyoma is the excessive deposition of ECM and key ECM components including collagens (Fig. 1), fibronectin, laminins and proteoglycans have been reported to be elevated in leiomyoma compared to myometrium (Stewart et al., 1994; Arici and Sozen, 2000; Norian et al., 2009; Malik et al., 2012).

Collagens

Collagen is an insoluble extracellular glycoprotein. It is a key structural component of ECM and maintains cellular morphology. In addition to their roles in wound healing and fibrosis, collagens are known to regulate cell migration, proliferation, differentiation and survival by signaling through cell surface receptors including integrins (Jones and Walker, 1999; Pickering, 2001). Leiomyoma cells have an increased expression of collagen subtypes and are organized by an abnormal collagen structure and orientation (Stewart et al., 1994; Leppert et al., 2004; Malik et al., 2010; Iwahashi and Muragaki, 2011). Stewart et al. reported overexpression of type I and III collagen mRNAs in leiomyoma compared to the adjacent myometrium (Stewart et al., 1994) while later studies confirmed an increase of type I and V collagen protein in leiomyoma compared to normal myometrium (Iwahashi et al., 2010; Iwahashi and Muragaki, 2011). Malik et al. measured levels of a series of collagen subtypes using microarray analysis and found that COL1A1, 4A2, 6A1, 6A2, 7A1 and 16A1 were each expressed to a greater extent in leiomyoma cells than in myometrial cells (Malik et al., 2010). Wolanska and co-scientists similarly reported that collagen concentration was higher in both small (defined as <10 g) and large (defined as >100 g) leiomyoma compared to normal myometrium (Wolanska et al., 1998). They also reported that small leiomyoma had a higher collagen concentration than large leiomyoma (Wolanska et al., 1998) suggesting that excessive accumulation of ECM components is an early event in the formation of these uterine leiomyoma.

Fibronectin

Like collagen, fibronectin is a secreted glycoprotein of the ECM and its role is to attach cells to a variety of ECM types (such as collagen, fibrin and heparin) through interacting with specific membrane receptors (such as integrins). Fibronectin is involved in cell migration, adhesion, growth and differentiation as well as fibrosis, tumor invasion and metastasis (Pankov and Yamada, 2002). Leiomyoma cells are reported to have an elevated level of fibronectin compared to myometrial cells (Arici and Sozen, 2000).

Laminins

Laminins are major glycoprotein components of ECM of the basal lamina (one of the layers of the basement membrane). They are composed of α-, β- and γ-subunits. Laminin has binding regions for collagen and fibronectin (Ockleford et al., 1993; Smith and Ockleford, 1994) and specific integrins also serve as cell surface receptors for laminins. Laminin plays an important role in cell migration, adhesion, growth and differentiation as well as assembly of ECM (Kleinman et al., 1985). Malik et al. demonstrated an increased expression of integrin β1 and integrin α6 subunit as well as laminin 5α, laminin 5β and laminin 5γ subunits in leiomyoma cells compared to myometrial cells (Malik et al., 2012). In addition, they plated leiomyoma cells in laminin coated culture plates, and found their alignment was parallel compared to plastic or collagen-I coated plates (Malik et al., 2012).

Fibulin-3

Fibulins are calcium-binding glycoproteins that associate with basement membranes and elastic fibers in the ECM. Fibulins may interact with fibronectin, laminins, proteoglycans and tropoelastin, and play important roles in cell morphology, growth, adhesion and motility (Gallagher et al., 2005). Fibulin-3 is encoded by the gene EFEMP1 (EGF-containing fibulin-like ECM protein 1). Marsh et al. demonstrated reduced expression of EFEMP1 and fibulin-3 in leiomyoma compared to normal myometrial cells and tissues by using multiple approaches, including real-time PCR, western blotting, immunohistochemistry and immunofluorescence (Marsh et al., 2016a). Since fibulin-3 acts as an antagonist of angiogenesis (Albig et al., 2006), downregulation of EFEMP1 and the associated loss of fibulin-3 protein may be associated with increased leiomyoma angiogenesis.

Proteoglycans

Proteoglycans are glycosylated proteins consisting of a 'core protein' with covalently attached glycosaminoglycans (GAGs). GAGs are long, unbranched heteropolysaccharide chains composed of repeating disaccharide units. The common GAGs are chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, heparin and hyaluronic acid. Berto et al. reported that the amounts of chondroitin sulfate and dermatan sulfate (combined data) were increased in leiomyoma compared to normal myometrial tissue (Berto

et al., 2001). In addition to the increases in chondroitin sulfate and dermatan sulfate (Berto et al., 2001; Mitropoulou et al., 2001), a rise in keratin sulfate was also found in leiomyoma relative to myometrial tissue (Wolanska et al., 1998). Wolanska et al. also reported elevations in heparan sulfate and heparin in leiomyoma relative to myometrial tissue (Wolanska et al., 1998). By contrast, however, a decrease in heparan sulfate concentration was reported in leiomyoma compared to myometrium in other studies (Berto et al., 2001; Mitropoulou et al., 2001). Hyaluronic acid is a large GAG and is unique in that it does not contain any sulfate and does not attach covalently to proteins. Available evidence suggests that expression levels of hyaluronic acid are not consistently higher or lower in leiomyoma than normal myometrium. For example, Wolanska et al. found no differences in the expression of hyaluronic acid in small leiomyoma compared to myometrial tissue (Wolanska et al., 1998), while another study found less hyaluronic acid in leiomyoma compared to normal myometrium (Mitropoulou et al., 2001). This apparent inconsistency may relate to the rather dynamic expression of proteoglycans in the uterus.

Versican is a large chondroitin sulfate proteoglycan that plays an important role in cell migration, adhesion, proliferation, tissue homeostasis and inflammation (Wight, 2002; Andersson-Sjöland et al., 2014). The expression of several versican subtypes was reported to be higher in leiomyoma tissue and primary cells compared to their healthy tissue counterparts by microarray analysis (Leppert et al., 2006; Malik and Catherino, 2007; Malik et al., 2010). Particularly, versican variant V0 was found to be dramatically elevated in leiomyoma (Malik et al., 2010). In addition, Carrino et al. reported higher amounts of versican in uterine leiomyoma and keloid scars compared to corresponding healthy tissues (Carrino et al., 2012), suggesting a molecular link in the ECM composition between these two fibrotic diseases.

Decorin is a small dermatan sulfate proteoglycan that regulates matrix assembly by binding to fibronectin and collagen via its core protein (Schmidt et al., 1991; Fiedler et al., 2008). Available evidence suggests that uterine leiomyoma contain less decorin than normal myometrial tissue (Carrino et al., 2012; Barker et al., 2015) and, since decorin may act as a antagonist of TGF-β signaling (Droguett et al., 2006), this may cause increased activation of TGF-β signaling that is important for fibrosis. Indeed, decorin treatment of several *in vivo* models of fibrosis was reported to ameliorate the fibrotic condition (Giri et al., 1997; Logan et al., 1999).

Fibromodulin is a small keratan sulfate proteoglycan that is widely expressed in many connective tissues. It has a close homology with decorin and biglycan. Fibromodulin binds to collagens, and influences the rate of fibrillogenesis (Hedbom and Heinegård, 1993; Font et al., 1998). Levens et al. reported that fibromodulin was highly expressed at mRNA and protein levels in leiomyoma compared to myometrial tissue (Levens et al., 2005).

MMPs and TIMPs

ECM remodeling is an essential process for development, wound healing and homeostasis as well as fibrosis. It is regulated by the combined action of MMPs and TIMPs. MMPs are a class of zinc-dependent endopeptidases that are responsible for the degradation of the ECM while TIMPs act as physiological regulators of the MMPs. Available evidence indicates that several MMPs and TIMPs are differentially expressed at both mRNA and protein level in leiomyoma compared to myometrium. Among the studied MMPs, the expression of MMP-1, -2, -3, -9, -11, -14, -16 and -24 is elevated, while the expression of MMP-7, -19 and -25 is decreased in leiomyoma (Palmer et al., 1998; Wolanska et al., 2004; Dimitrova et al., 2009; Malik et al., 2010; Tsigkou et al., 2015) (Table I). Particularly, the activity and circulating level of MMP-2 are elevated in leiomyoma compared to normal myometrium (Wolanska et al., 2004; Bogusiewicz et al., 2007; Korompelis et al., 2015), indicating a possible dominant role of this MMP in ECM remodeling in leiomyoma. The current research suggests that MMPs are not only ECM-degrading proteases; they participate in a wide range of physiological processes including cell migration, differentiation, growth, innate and adaptive immunity, inflammation, angiogenesis, apoptosis, bone remodeling and neurite growth (Nagase et al., 2006; Rodríguez et al., 2010; Löffek et al., 2011). Some MMPs are antifibrotic, whereas others can have profibrotic functions (Giannandrea and Parks, 2014) (Table I). They can directly or indirectly affect the functions of various cytokines that play roles in inflammation and repair processes including interferon-β (Nelissen et al., 2003), VEGF (Bergers et al., 2000), EGF (Suzuki et al., 1997), FGF (Suzuki et al., 1997) and TGF-β1 (Shull et al., 1992). MMPs can regulate the bioavailability of angiogenic factors sequestered by the ECM. For instance, ECM-bound VEGF is mobilized by MMPs-1, -3, -7, -9, -16 and -19 cleavage of the VEGF-binding ECM proteins (Bergers et al., 2000; Colnot et al., 2003; Lee et al., 2005). MMPs also enable proteolytic release of different ECM-trapped growth factors such as FGFs or TGF-β that can show strong mitogenic properties (Rodríguez et al., 2010). MMPs can both activate or prevent apoptosis through proteolytic processing of particular signaling molecules. For example, the Fas/Fas ligand (FasL) signal transduction axis is a vital pro-apoptotic system affected by MMPs. MMP-7-mediated release of soluble FasL triggers apoptosis in epithelial cells while inhibiting cell death in tumors (Powell et al., 1999b; Mitsiades et al., 2001). MMP-7 also inhibits apoptosis in cancer cells by cleaving Fas (Strand et al., 2004). Regarding TIMPs, Bogusiewicz et al. reported that the levels of TIMP-1 and TIMP-2 were similar in both myometrial and leiomyoma tissues (Bogusiewicz et al., 2007) while circulating levels of TIMP-1 were reported to be significantly elevated in leiomyoma patients compared to controls (Korompelis et al., 2015). Overall, the available evidence suggests a dysregulation of MMPs and TIMPs that may play a critical role in the formation of a more fibrous ECM in leiomyoma.

Mechanotransduction of the extracellular matrix

Mechanotransduction is a process that allows cells to adapt to their changing physical surroundings by sensing their environment and translating mechanical stress into biochemical signals (Huang et al., 2004; Thorne et al., 2015). This process is promoted by the stiffness of the ECM or osmotic stress that initiates cell-matrix interactions in a rapid, transient and bidirectional manner which is referred to as 'dynamic reciprocity' (Bissell and Aggeler, 1986; Maniotis et al., 1997; Polacheck et al., 2014; Thorne et al., 2015) (Fig. 2). The activation of downstream mechanical signaling pathways can alter gene expression, leading to changes in ECM density, composition and organization that

Table I Expression of MMPs and TIMPs in uterine leiomyoma compared to myometrium.

MMMs and TIMPs	Expression in leiomyoma relative to myometrium	Matrix substrates/MMPs	Functions in different biological systems
MMP-1	↑	Collagen types I, II, III, VII and X as well as gelatin, entactin, aggrecan and tenascin (McCawley and Matrisian, 2001)	(I) Plays an antifibrotic role in liver fibrosis (Iimuro *et al.*, 2003) (II) Possesses pro-apoptotic effects (Mannello *et al.*, 2005)
MMP-2	↑	Fibronectin, laminin, gelatin, elastin, aggrecan and vitronectin as well as collagen types I, IV, V, VII, X and XI (McCawley and Matrisian, 2001)	(I) Plays an antifibrotic role in liver fibrosis (Onozuka *et al.*, 2011) (II) Promotes angiogenesis (Webb *et al.*, 2017) (III) Possesses both pro-apoptotic and anti-apoptotic effects (Mannello *et al.*, 2005)
MMP-3	↑	Fibronectin, laminin, proteoglycans, gelatins, fibrinogen, entactin, tenascin and vitronectin as well as collagen types III, IV, V and IX (McCawley and Matrisian, 2001)	(I) Appears to be profibrotic in lung fibrosis (Yamashita *et al.*, 2011) (II) Appears to play a role in angiogenesis (Anuar *et al.*, 2016) (III) Possesses both pro-apoptotic and anti-apoptotic effects (Mannello *et al.*, 2005)
MMP-7	↓	Collagen types III, IV, V, IX, X and XI as well as proteoglycans, laminin, fibronectin, gelatin, fibrinogen, entactin, tenascin and vitronectin (McCawley and Matrisian, 2001)	(I) Plays a profibrotic role in lung fibrosis (Manicone *et al.*, 2009) (II) Induces angiogenesis (Nishizuka *et al.*, 2001) (III) Possesses both pro-apoptotic and anti-apoptotic effects (Mannello *et al.*, 2005)
MMP-9	↑	Elastin and gelatin as well as collagen types IV and V (Matrisian, 1992)	(I) Possesses both anti- and profibrotic functions in lung fibrosis (Lee *et al.*, 2001; Cabrera *et al.*, 2007) (II) Promotes angiogenesis (Webb *et al.*, 2017) (III) Possesses both pro-apoptotic and anti-apoptotic effects (Mannello *et al.*, 2005)
MMP11	↑	Laminin, fibronectin and aggrecan (McCawley and Matrisian, 2001)	(I) Shows dual functions in tumourigenesis and cancer progression (Zhang *et al.*, 2016) (II) Possesses both pro-apoptotic and anti-apoptotic effects (Mannello *et al.*, 2005)
MMP-14	↑	Collagen, fibronectin, gelatin, vitronectin, tenascin, nidogen, aggrecan, fibrin, fibrinogen and laminin-5 (McCawley and Matrisian, 2001; Klein and Bischoff, 2011)	(I) Shows diverse and opposing functions in scarring or fibrosis (Rohani and Parks, 2015) (II) Promotes cell migration (Itoh, 2006) (III) Promotes tumor growth and angiogenesis (Sounni *et al.*, 2002)
MMP-16	↑	Collagen type III, gelatin and fibronectin (Matsumoto *et al.*, 1997)	(I) promotes cancer progression (Sounni and Noël, 2005)
MMP-19	↓	Fibronectin, tenascin-C, gelatin, nidogen and collagen type IV (Stracke *et al.*, 2000)	(I) possesses both anti- and profibrotic functions in liver fibrosis (Wang *et al.*, 2011; Jirouskova *et al.*, 2012) (II) exhibits an anti-angiogenic effect on endothelial cells (Brauer *et al.*, 2011)
MMP-24	↑	Fibronectin, gelatin, vitronectin, collagen and aggregan (McCawley and Matrisian, 2001)	(I) Plays an important role in ECM remodeling events in the brain and during embryonic development (Pei, 1999)
MMP-25	↓	Fibronectin, collagen type IV gelatin, fibrin and laminin-1 (McCawley and Matrisian, 2001)	() Appears to play a role in cancer progression (Velasco *et al.*, 2000; Sun *et al.*, 2007)
TIMP-1	~	Collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, MMP-11 and MMP-18), matrilysins (MMP-7, MMP-26), membranous MMP (MMP-15, MMP-16, MMP-17, MMP-24 and MMP-25), and others (MMP-11, MMP-12, MMP-19, MMP-20, MMP-23 and MMP-28) (Jakubowska *et al.*, 2016)	(I) Promotes liver fibrosis (Yoshiji *et al.*, 2000) (II) Inhibits apoptosis (Li *et al.*, 1999) (III) Inhibits tumor growth and angiogenesis (Ikenaka *et al.*, 2003)
TIMP-2	~	Collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, MMP-11 and MMP-18), matrilysins (MMP-7, MMP-26), membranous MMP (MMP-14, MMP-	(I) Promotes survival of hepatic stellate cells and endothelial cells (Murphy *et al.*, 2002; Boulday *et al.*, 2004)

Continued

Table I *Continued*

MMMs and TIMPs	Expression in leiomyoma relative to myometrium	Matrix substrates/MMPs	Functions in different biological systems
		15, MMP-16, MMP-17, MMP-24 and MMP-25), and others (MMP-11, MMP-12, MMP-19, MMP-20, MMP-23 and MMP-28) (Jakubowska *et al.*, 2016)	(II) Inhibits endothelial cell migration and angiogenesis (Seo *et al.*, 2003; Oh *et al.*, 2004)

ultimately affect cell shape and contractility (Fig. 2). Cells sense the mechanical force from newly generated altered ECM and further activate mechanical signaling that result in altered cell behavior and overall remodeling of the matrix. This process appears to play a crucial role in cell adhesion, migration, proliferation and survival as well as inflammation and fibrosis (Seong *et al.*, 2013; Duscher *et al.*, 2014; Thorne *et al.*, 2015). Mechanotransduction occurs at the sites of focal adhesion (Seong *et al.*, 2013; Mui *et al.*, 2016) where large protein complexes serve as a 'molecular bridge' between ECM and intracellular molecules/cytoskeleton (Geiger *et al.*, 2001). At these sites, mechanical signals are transmitted from the ECM to the intracellular space through heterodimeric (α and β) transmembrane integrins. Upon ligation and clustering, integrins activate cytoplasmic tyrosine kinases including Src and focal adhesion kinase (FAK). Activation of FAK results in the recruitment of a number of SH2 (Src Homology 2) domain- and SH3 domain-containing proteins that mediate downstream signaling pathways (Parsons, 2003; Schlaepfer and Mitra, 2004) including ERK 1/2, p38 MAPK and JNK (c-Jun N-terminal kinase) (Ruwhof and van der Laarse, 2000; PaszeK *et al.*, 2005). Rho is one of the targets of FAK (Hanks *et al.*, 1992). The Rho family of GTPases, include Rac, Cdc42 and RhoA (Ras homolog gene family, member A). RhoA serves as a 'molecular switch' that mediates cycling between active GTP (guanosine triphosphate)-bound and inactive GDP (guanosine diphosphate)-bound states. It plays a crucial role in mediating several profibrotic responses in cardiac fibroblasts (Porter *et al.*, 2004; Zhao *et al.*, 2007). A kinase anchor protein 13 (AKAP13), a RhoA GTPase-specific guanine exchange factor (Rho-GEF), is known to activate RhoA from the inactivated form to the activated RhoA GTPase. The AKAP13/RhoA complex was recently reported to mediate the profibrotic effects in cardiac fibroblasts (Cavin *et al.*, 2014). In addition, AKAP13 appears to play a key role in transmitting the osmotic stress or extracellular hyperosmolarity signal through nuclear factor of activated T cells 5 (NFAT5) (Kino *et al.*, 2009). The cellular osmotic response results in fluid exchange between the cell and ECM that may affect cell shape and ECM composition and organization (Polacheck *et al.*, 2014).

Uterine leiomyoma has been reported to be significantly stiffer than matched myometrium (Rogers *et al.*, 2008), and appears to be under increased mechanical stress (Rogers *et al.*, 2008). In leiomyomal cells, the levels of integrin α6 and integrin β1 were found to be overexpressed compared to normal counterparts (Malik *et al.*, 2012; Chen *et al.*, 2013). In addition, phosphorylation of FAK and ERK was increased in leiomyomal cells (Malik *et al.*, 2012; Chen *et al.*, 2013). The upstream target of ERK, RhoA was overexpressed in leiomyoma compared to myometrial cells (Norian *et al.*, 2012). Malik *et al.* reported that inhibition of integrin-β1 led to a decrease in active RhoA in leiomyoma cells (Malik *et al.*, 2012). Fasudil, an inhibitor of Rho kinase (ROCK, a downstream target of RhoA), was found to relax the contraction of leiomyoma cells in 3D collagen gels (Malik *et al.*, 2014). The levels of AKAP13 (a Rho-GEF) also appeared higher in leiomyoma compared to myometrium (Rogers *et al.*, 2008). Rogers and co-investigators further reported that p38 MAPK phosphorylation was greater in leiomyoma compared to matched myometrium (Rogers *et al.*, 2008), and this increase was associated with upregulation of AKAP13 (Rogers *et al.*, 2008).

NFAT5 is a transcription factor that induces the expression of genes involved in osmotic stress. Uterine leiomyoma cells demonstrated increased basal expression of NFAT5 compared to myometrial cells. The expression of NFAT5 as well as NFAT5-regulated genes, AR (aldose reductase) and SMIT (sodium myo-inositol transporter 1) was further increased in leiomyoma cells under hyperosmolar conditions (McCarthy-Keith *et al.*, 2011).

A recent study suggested that collagen may play a role in smooth muscle cell proliferation in uterine leiomyoma (Koohestani *et al.*, 2013). Using an *in vitro* model system of collagen, Koohestani *et al.* investigated the interaction of cultured leiomyoma smooth muscle cells with monomeric unpolymerized collagen films and fibrillar polymerized collagen gels, and found a significant increase in cell proliferation (Koohestani *et al.*, 2013). The differences in cell proliferation were accompanied by changes in cell cycle progression and p21 (Koohestani *et al.*, 2013). The overall result suggests a possible activation of ECM mediated integrin-FAK-Rho-ERK-p38 MAPK-signaling leading to cell proliferation. Indeed, Malik and co-investigators reported that inhibition of integrin β1 led to a decrease in phosphorylation of ERK as well as reduced proliferation of leiomyoma cells (Malik *et al.*, 2012).

Regulation of extracellular matrix accumulation by growth factors and cytokines

As discussed below, several growth factors and cytokines regulate the production of ECM in leiomyoma cells and can contribute to fibrosis (Islam *et al.*, 2013a, 2016) (Fig. 3).

Transforming growth factor-β

Transforming growth factor-β (TGF-β) is a secreted polypeptide with multifunctional properties. It has three isoforms, referred to as TGF-β1, TGF-β2 and TGF-β3. Myometrial and leiomyoma smooth muscle cells express mRNAs and proteins for each of the three TGF-β

isoforms as well as for the TGF-β receptors, TGF-βR-I and TGF-βR-II (Dou et al., 1996; Tang et al., 1997). TGF-β3 appears to increase mRNA expression of ECM components, including collagen 1A1, connective tissue growth factor (CTGF) (Joseph et al., 2010), fibronectin (Arici and Sozen, 2000) and versican V0 (Norian et al., 2009) in both myometrial and leiomyoma cells. However, TGF-β3 was reported to decrease production of MMP-2 and MMP-11 in myometrial and leiomyoma cells (Joseph et al., 2010). In addition to TGF-β3, TGF-β1 has been reported to increase mRNA expression of CTGF (Luo et al., 2006), plasminogen activator inhibitor 1 (PAI-1) (Ding et al., 2004b) and fibromodulin (Levens et al., 2005) in myometrial and leiomyoma smooth muscle cells. The profibrotic role of TGF-β is mediated, at least in part, through activation of Smad 2/3 and ERK 2/3 signaling pathways (Xu et al., 2003; Ding et al., 2004b).

Activin-A

Activin-A, a member of the TGF-β superfamily, was originally isolated for its ability to stimulate secretion of FSH from the anterior pituitary gland but is now known to have wide-ranging physiological and pathophysiological roles in multiple tissues. The mRNA level of activin-A was found to be more highly expressed in leiomyoma compared to myometrium, whereas the levels of activin receptors (ALK4, ActRIIA and ActRIIB) were unchanged (Ciarmela et al., 2011a). The profibrotic role of activin-A in leiomyoma cells has recently been demonstrated by our group (Islam et al., 2014a). We found that activin-A can increase mRNA levels of several ECM components, including collagen 1A1, fibronectin and versican in human primary leiomyoma cells compared to untreated controls (Islam et al., 2014a). Furthermore, activin-A can induce phosphorylation of Smad2 and Smad3 in both myometrial and leiomyoma cells compared to untreated cells (Islam et al., 2014a), suggesting that the fibrotic role of activin-A is mediated, at least in part, by activation of Smad 2/3 signaling pathway.

Platelet-derived growth factor

PDGF is a family of growth factors consisting of four homodimers, namely PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD, and one heterodimer, PDGF-AB. The protein levels of PDGF-AA, PDGF-BB and PDGF-CC and their receptors were found to be highly expressed in leiomyoma compared to myometrial cells and/or tissues (Liang et al., 2006; Hwu et al., 2008; Yu et al., 2008; Suo et al., 2009). PDGF exerts a profibrotic effect in myometrial and leiomyoma cells through increasing collagen α1 (I) expression (Liang et al., 2006). Additionally, PDGF can stimulate myometrial and leiomyoma cell proliferation (Liang et al., 2006; Mesquita et al., 2010) by regulating of VEGF production (Taniguchi et al., 2001) and activating the ERK 1/2 signaling pathway (Mesquita et al., 2010).

Tumor necrosis factor-α

Tumor necrosis factor (TNF)-α is a pleiotropic cytokine that is primarily secreted by activated macrophages. It plays an important role in controlling inflammation, immunity, cell growth and differentiation, and apoptosis. The level of TNF-α protein expression was found to be higher in leiomyoma compared to normal myometrial cells and tissues (Kurachi et al., 2001; Plewka et al., 2013). Wang et al. reported that TNF-α can significantly upregulate the protein and mRNA levels of MMP-2 in cultured leiomyoma smooth muscle cells but not in matched myometrial smooth muscle cells (Wang et al., 2015). Recently, we found that TNF-α can increase activin-A mRNA expression in both myometrial and leiomyoma cells (Islam et al., 2013b). The increased expression of activin-A in leiomyoma (Ciarmela et al., 2011a), and the ability of activin-A is to increase production of collagen 1A1, fibronectin and versican in leiomyoma smooth muscle cells (Islam et al., 2014a), supports the possible role of TNF-α and activin-A in the process of fibrosis following leiomyoma growth. Additionally, Nair and Al-Hendy demonstrated the possible of role of TNF-α in human uterine leiomyoma cell proliferation (Nair and Al-Hendy, 2011). The proliferative and profibrotic effect of TNF-α in leiomyoma cells was mediated, at least in part, by activating ERK 1/2 signaling pathway (Wang et al., 2015).

Other cytokines

Several cytokines, including interleukin (IL)-1β, IL-11, IL-13 and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been reported to be highly expressed in leiomyoma (Chegini et al., 1999; Ding et al., 2004a; Luo et al., 2005; Syssoev et al., 2008; Plewka et al., 2013). Since these cytokines are known to play important roles in the development of fibrosis in different cellular systems (Xing et al., 1997; Chakir et al., 2003; Fichtner-Feigl et al., 2005; Guo et al., 2013), they may have fibrotic roles in the pathogenesis of leiomyoma.

Hormonal regulation of extracellular matrix accumulation

It is well accepted that estrogen and progesterone play prominent roles as regulators of uterine leiomyoma growth (Maruo et al., 2004; Ciarmela et al., 2011b; Islam et al., 2013a). However, the underlying mechanisms are not completely understood. A number of studies suggest that the stimulatory effects of estrogen and progesterone on leiomyoma growth are mediated, at least in part, by regulation of ECM proteins, growth factors and their signaling pathways (Barbarisi et al., 2001; Chegini et al., 2002; Hoekstra et al., 2009; Nierth-Simpson et al., 2009; Qiang et al., 2014; Barker et al., 2015) (Fig. 3).

Estrogen

Estrogens exert multiple effects on their target cells through binding to estrogen receptors (ERα and ERβ) (Nilsson et al., 2001). Uterine leiomyoma exhibits higher mRNA and protein expression levels of ERα (more highly expressed) and ERβ than normal myometrium (Benassayag et al., 1999; Kovacs et al., 2001). Particularly, ER-α and ER-β are more highly expressed at both mRNA and protein levels in leiomyoma fibroblasts compared to smooth muscle fibroblasts (Feng et al., 2016). Using primary cultures, Zbucka et al. found that collagen biosynthesis was strongly stimulated by low doses of estrogen (5 nM) in leiomyoma cells while no changes occurred in myometrial cells (Zbucka et al., 2008). In contrast, collagen production was inhibited by 10 nM estrogen in both leiomyoma as well as control cells (Zbucka et al., 2008). In uterine leiomyoma xenografts, 17β-estradiol

was found to upregulate mRNAs for multiple collagens through downregulation of miR-29b (Qiang *et al.*, 2014). Recently, higher expression of collagen I, fibronectin and laminin, were found in estrogen-treated leiomyoma and smooth muscle fibroblasts compared to untreated control groups (Feng *et al.*, 2016). However, Zbucka *et al.* found that estrogen can inhibit MMP-2 in leiomyoma and myometrial cells (Zbucka *et al.*, 2008) while transfection of a dominant-negative estrogen receptor resulted in significant augmentation of MMP-1 protein expression in primary and immortalized human leiomyoma cultures (Hassan *et al.*, 2007). Chegini and co-investigators demonstrated that estradiol stimulated both total and active TGF-β1 production in both leiomyoma and myometrial smooth muscle cells (Chegini *et al.*, 2002). The secretion of TGF-β and IGF-1 was also found to be significantly increased in response to estrogen treatment in both leiomyoma and smooth muscle fibroblasts compared to untreated controls (Feng *et al.*, 2016). Recently, we found that estrogen can downregulate activin-A mRNA expression in human myometrial explants (Ciarmela *et al.*, 2011a). Barbarisi *et al.* reported that estrogen can upregulate PDGF expression in cultured leiomyoma cells (Barbarisi *et al.*, 2001). The ability of estrogen to induce PDGF expression was further confirmed by the observation by Barbarisi *et al.* that an antiestrogenic compound ICI 182 780 prevented estrogen-induced PDGF expression in cultured leiomyoma cells (Barbarisi *et al.*, 2001). They further reported rapid and transient activation of the MAPK pathway in estrogen-treated leiomyoma smooth muscle cells compared to untreated groups (Barbarisi *et al.*, 2001). Treatment of leiomyoma smooth muscle cells with estrogen was also reported to increase phosphorylation of several downstream intracellular proteins, such as GAP (GTPase-activating protein), PI3K, PLCγ (phospholipase-Cγ) and ERK 1/2 (Barbarisi *et al.*, 2001; Nierth-Simpson *et al.*, 2009). A recent study reported that estrogen can also promote phosphorylation of MEK, ERK1/2 and AKT (also known as protein kinase B or PKB) in both leiomyoma and smooth muscle fibroblasts compared to untreated controls (Feng *et al.*, 2016).

Progesterone

Progesterone exerts physiologic actions on its target cells by interacting with progesterone receptors (PR-A and PR-B) (Kastner *et al.*, 1990). Progesterone seems to be the dominant steroidal influence on uterine leiomyoma as indicated by the increased mitotic rates of leiomyoma cells during the secretory phase of the menstrual cycle (Kawaguchi *et al.*, 1989). Recently, Barker *et al.* tested the effects of progesterone on decorin expression in human uterine leiomyoma compared with normal myometrial tissue from different stages of the menstrual cycle (Barker *et al.*, 2015). This group found lower expression of decorin at both the mRNA and protein levels in leiomyoma than in adjacent myometrium (Barker *et al.*, 2015). They also found reduced level of decorin mRNA expression in leiomyoma tissue from the secretory phase compared to the proliferative phase (Barker *et al.*, 2015), suggesting an inhibitory effect of progesterone on decorin expression in leiomyoma. As expected, progesterone was found to decrease mRNA expression of decorin in uterine leiomyoma cells compared to controls (Barker *et al.*, 2015). Since decorin inhibits the activity of TGF-β (Yamaguchi *et al.*, 1990), its reduced level in leiomyoma may enhance ECM deposition through TGF-β activity. Chegini *et al.* demonstrated that treatment of primary myometrial and leiomyoma cell cultures with medroxyprogesterone acetate, a synthetic variant of the steroid hormone progesterone, stimulated both total and active TGF-β1 production (Chegini *et al.*, 2002). Consistently, the highest level of TGF-β3 mRNA was observed in leiomyoma from the mid-secretory phase of menstrual cycle (Arici and Sozen, 2000). Hoekstra *et al.* reported that progestins can rapidly increase the levels of phosphorylated-AKT and its downstream effectors, pGSK3β and pFOXO1 in leiomyoma cells (Hoekstra *et al.*, 2009). Recently, ECM regulation by progesterone via miRNA was reported by Qiang and co-workers (Qiang *et al.*, 2014). This group found a lower level of miR-29b in uterine leiomyoma compared to myometrial tissues as well as primary cells (Qiang *et al.*, 2014). The restoration of miR-29b in the uterine leiomyoma cells reduced the COL1A1 level in cultured uterine leiomyoma cells. In uterine leiomyoma xenografts, restoring miR-29b also inhibited the accumulation of several collagen subtypes and the development of solid tumors (Qiang *et al.*, 2014). This group demonstrated that progesterone can upregulate mRNA expression of collagens via downregulating miR-29b in a leiomyoma xenograft model (Qiang *et al.*, 2014).

Regulation of extracellular matrix accumulation by microRNA

MicroRNAs (or miRNAs) are small non-coding RNAs (~22-nt long) that regulate post-transcriptional gene expression. By binding to the 3'-UTR (untranslated region) of target mRNAs, miRNAs prevent protein production by inducing mRNA degradation and/or directly repress translation. A significant number of studies have suggested that miRNAs play an important role in the regulation of ECM accumulation during leiomyoma pathogenesis (Wang *et al.*, 2007; Luo and Chegini, 2008; Chuang *et al.*, 2012a, 2012b; Chuang and Khorram, 2014; Qiang *et al.*, 2014; Chuang and Khorram, 2016; Marsh *et al.*, 2016b).

miR-29 family

miR-29a

Uterine leiomyoma has been demonstrated to have decreased levels of miR-29a in leiomyoma relative to myometrium (Marsh *et al.*, 2016b). Marsh's group used Ambion miRNA precursors to successfully overexpress miRNA-29 in cell culture, and demonstrate its role in ECM production. They found that overexpression of miRNA-29a causes a decrease in the production of collagen subtypes (types II and III) in leiomyoma cells compared to control cells (Marsh *et al.*, 2016b). However, the knockdown of miRNA-29 failed to impact collagen expression in leiomyoma cells compared to control cells (Marsh *et al.*, 2016b).

miR-29b

Uterine leiomyoma expressed a lower level of miR-29b than myometrium (Wang *et al.*, 2007; Qiang *et al.*, 2014; Marsh *et al.*, 2016b) and forced expression of miR-29b reduced the COL1A1, COL2A1 and COL3A1 protein level in primary leiomyoma cells (Qiang *et al.*, 2014; Marsh *et al.*, 2016b). In leiomyoma xenografts, overexpression of miR-

29b also inhibited the accumulation of collagens (COL1A1 and COL1A3) and the development of solid tumors (Qiang et al., 2014). However, knockdown of miR-29b in myometrium xenografts increased the expression of collagens (COL1A1, COL1A2, COL3A1, COL5A1, COL5A3 and COL7A1) without transformation into leiomyoma (Qiang et al., 2014). This result suggests that the downregulation of miR-29b is essential but not sufficient for leiomyoma tumourigenesis. Qiang's group reported that estrogen and progesterone can downregulate miR-29b, and upregulate collagen expression in leiomyoma xenografts (Qiang et al., 2014), indicating that steroid hormones promote leiomyoma growth, at least in part, through increasing ECM production via downregulation of the tumor suppressor miR-29b.

miR-29c

Similar to miR-29a and miR-29b, miR-29c is also expressed at low levels in leiomyoma compared to myometrium (Luo and Chegini, 2008; Chuang and Khorram, 2016; Marsh et al., 2016b). Levels of miR-29c were inversely associated with expression of its target, COL3A1 (Chuang and Khorram, 2016) and gain of function of miR-29c inhibited the protein and mRNA expression of COL3A1, and reduced secreted COL3A1, and the rate of cell proliferation (Chuang and Khorram, 2016). Marsh *et al.* also reported that overexpression of miRNA-29c decreased protein expression of the major collagen subtypes (COL1A1, COL2A1 and COL3A1) in primary leiomyoma cells compared to control cells (Marsh et al., 2016b). The suppression of miR-29c in leiomyoma smooth muscle cells was primarily mediated by steroid hormones, NF-κB and SP1transcription factors, and DNA methylation (Chuang and Khorram, 2016).

miR-200c

Uterine leiomyoma expresses very low levels of miR-200c relative to myometrium (Chuang et al., 2012b; Chuang and Khorram, 2014). Gain-of-function of miR-200c repressed the expression of ZEB1 and ZEB2, TIMP2 and FBLN5 (fibulin 5) at the mRNA and protein levels in leiomyoma and/or smooth muscle cells (Chuang et al., 2012b). Gain-of-function of miR-200c in leiomyoma smooth muscle cells also reduced mRNA and protein levels of IL8 (an inflammatory mediator) (Chuang and Khorram, 2014). In contrast, knockdown of miR-200c produced the opposite effect with a significant increase in IL8 mRNA in these cells (Chuang and Khorram, 2014). This regulation was mediated by suppressing the NF-κB pathway through targeting IKBKB (Chuang and Khorram, 2014).

miR-93/106b

Uterine leiomyoma expresses significantly lower levels of miR-93, but not miR-106b, as compared with myometrium (Chuang et al., 2012a). The gain of function of miR-93 and miR-106b in leiomyoma smooth muscle cells and myometrial smooth muscle cells repressed mRNA and protein levels of tissue factor (F3) and IL8 at through direct interactions with their respective mRNA3'-UTRs (Chuang et al., 2012a) and indirectly inhibited IL8, CTGF and PAI-1 expression through F3 repression (Chuang et al., 2012a).

Extracellular matrix acts as a reservoir for growth factors and modulator of their actions

An elegant experimental *in vitro* co-culture model by Moore and coworkers has demonstrated that leiomyoma-derived fibroblasts enhance collagen type I production, activate receptor tyrosine kinases (RTKs) and TGF-β signaling and stimulate leiomyoma cell proliferation (Moore et al., 2010). They also found an increased secretion of several growth factors, including IGF, PDGF, TGF-β, EGF and FGF in the ECM of cultured uterine leiomyoma cells (Moore et al., 2010), suggesting an important link among ECM, growth factors, their receptors and signaling pathways in the pathogenesis of leiomyoma.

Uterine leiomyoma is reported to express several GAG components of proteoglycans, such as heparan sulfate, heparin and keratan sulfate (Wolanska et al., 1998; Berto et al., 2001; Mitropoulou et al., 2001). Among these, heparin or heparan sulfate is known to bind to many growth factors. For example, heparan sulfate can bind to bFGF as a cofactor to facilitate the formation and signaling of bFGF-bFGF receptor complexes (Rapraeger et al., 1991; Yayon et al., 1991). Endothelial cell-derived heparan sulfate can also bind to bFGF and protect it from proteolytic degradation (Saksela et al., 1988), effectively serving as a storage depot for this growth factor. bFGF is commonly known as an inducer of angiogenesis and uterine leiomyoma has an increased expression of bFGF in leiomyoma compared to myometrium (Mangrulkar et al., 1995; Wolanska and Bankowski, 2006). FGFR-1 and FGFR-2 receptor expression levels were also found to be elevated in leiomyoma compared to myometrium (Wolanska and Bankowski, 2006; Yu et al., 2008). bFGF was found to be primarily bound to the ECM of myometrium and leiomyoma (Mangrulkar et al., 1995; Dixon et al., 2000). Particularly, leiomyoma showed stronger staining for bFGF because of large areas of ECM in these tumors (Mangrulkar et al., 1995; Dixon et al., 2000). ECM of uterine leiomyoma may therefore serve as a reservoir for bFGF.

A direct physical interaction between VEGF and heparan sulfate has been reported (Ortéga et al., 1998) and VEGF can bind to the heparin-II domain of fibronectin (Wijelath et al., 2006). The activation of VEGFR-2 can induce tyrosine phosphorylation of integrin β3 (Mahabeleshwar et al., 2007). Thus, the relationship between VEGFR-2 and integrin β3 seems to be synergistic. Uterine leiomyoma and myometrium were both reported to express VEGFR-1 and VEGFR-2 (Harrison-Woolrych et al., 1995; Brown et al., 1997; Gentry et al., 2001; Sanci et al., 2011). However, VEGF-A protein levels were found to be higher in leiomyoma than in adjacent myometrium (Gentry et al., 2001). Hassan *et al.* demonstrated that VEGF was required for continuous growth of tumors or leiomyoma in a xenograft mouse model (Hassan et al., 2008).

Growth factors can also bind to heparan sulfate indirectly through accessory proteins. For example, an activin-binding protein, follistatin, associates with heparan sulfate in follicular granulosa cells (Nakamura et al., 1991). Similarly, TGF-β binds to heparan sulfate via a 60 kDa protein (Bützow et al., 1993). In certain growth factors, such as PDGF-A and VEGF, the major heparin-binding site is localized to a linear 20 amino acid stretch rich in basic residues. The presence of heparan sulfate binding sequences in PDGF-A and VEGF can be modulated by alternative splicing (Raines and Ross, 1992; Park et al., 1993).

The interaction between TGF-β and the core protein of the proteoglycan, decorin has been reported (Border *et al.*, 1990; Yamaguchi *et al.*, 1990). TGF-β1 can also induce the synthesis of decorin by mesangial cells (Border *et al.*, 1990). Decorin, in turn, binds to TGF-β and acts a ligand trap that blocks its ability to bind its receptors on target cells (Yamaguchi *et al.*, 1990), suggesting a negative feedback loop. Uterine leiomyoma tissue contains less decorin protein than normal myometrial tissues (Carrino *et al.*, 2012), suggesting a mechanism for increased activation of TGF-β signaling. Collagen type II contains a chordin-like VWC domain which can also bind to TGF-β1 (Abreu *et al.*, 2002) and act as a negative regulator for this growth factor. By contrast, a membrane-anchored proteoglycan, betaglycan (TGF-β type III receptor) was reported to facilitate the formation of TGF-β receptors complexes (Shi and Massagué, 2003). TGF-β binds first to betaglycan and that binding and 'presentation' play key roles in mediating TGF-β binding to the type II receptor (Shi and Massagué, 2003).

A hallmark of the integrins is the ability to recognize multiple ligands. They can activate growth factor mediated signaling pathways independently (Assoian and Schwartz, 2001) or synergistically with other receptors (Alam *et al.*, 2007). These include TGF-β receptors (Scaffidi *et al.*, 2004), VEGF receptors (Mahabeleshwar *et al.*, 2007), PDGF-β receptors (Schneller *et al.*, 1997), insulin-like growth factor receptors (IGFR) (Doerr and Jones, 1996), and EGF receptors (Bill *et al.*, 2004). In addition to integrins, several ECM proteins, including laminin, fibrillin, tenascin and thrombospondin contain EGF-like domains, which can directly bind to EGF receptors and modulate their signaling (Schenk *et al.*, 2003).

Fibrosis in uterine leiomyoma: role of myofibroblasts and inflammation

Fibrosis is caused by an excessive accumulation of ECM proteins resulting from an imbalance in wound healing and repair processes during chronic tissue injury and/or inflammation (Wynn, 2007). Myofibroblasts are specialized cell type, activated by tissue injury, inflammation, hypoxia and oxidative stress, that play critical roles in wound healing and tissue homeostasis as well as fibrosis (Poli, 2000; Higgins *et al.*, 2007; Wynn, 2007; Toullec *et al.*, 2010) (Fig. 1). A biological function of myofibroblasts is to produce ECM proteins in order to heal wounds and maintain functional integrity of organs/tissues after injury. After finishing this role in tissue repair and homeostasis, myofibroblasts disappear via apoptosis (Desmouliere *et al.*, 1995). When this process fails to proceed normally, persistence of cells with myofibroblastic phenotype can lead to excessive ECM production and development of fibrosis (Powell *et al.*, 1999a; Tomasek *et al.*, 2002) (Fig. 1).

Myofibroblast cells are characterized by α-SMA expression and a high level of collagen synthesis (Wynn, 2008). The fibronectin domain ED-A is also crucial for the myofibroblastic phenotype (Serini *et al.*, 1998). Several cell types, including fibroblasts, fibrocytes, stem cells, smooth muscle cells and endothelial cells have been reported to acquire the myofibroblast phenotype in large variety of organs (Hinz *et al.*, 2007) and a number of studies suggest that myofibroblastic transformation could occur from fibroblasts, stem cells and smooth muscle cells in the myometrium (Ono *et al.*, 2007; Chang *et al.*, 2010; Moore *et al.*, 2010; Holdsworth-Carson *et al.*, 2014; Zheng *et al.*, 2014; Mas *et al.*, 2015; Yin *et al.*, 2015; Feng *et al.*, 2016; Protic *et al.*, 2016) (Fig. 1). CD90, a fibroblast specific marker, was found to be expressed in both smooth muscle leiomyoma fibroblasts but not in smooth muscle cells themselves (Luo *et al.*, 2014). In addition, FAP (fibroblast activation protein), a marker of active fibroblasts, was highly expressed at the protein level in fibroblasts from uterine leiomyoma compared to those from normal muscle tissues (Luo *et al.*, 2014). Recently, we identified numerous CD34+ fibroblasts in myometrium and leiomyoma, which are known to give origin to myofibroblasts when they lose CD34 expression (Protic *et al.*, 2016). In addition, Yin *et al.* identified human uterine leiomyoma stem/progenitor cells expressing CD34 and CD49b, which are able to initiate tumors in vivo (Yin *et al.*, 2015). In our recent study, we reported the presence of α-SMA positive and desmin negative cells as well as large amount of collagen inside the leiomyoma tissue that supports the existence of myofibroblast and fibrotic characters of this tumor (Protic *et al.*, 2016).

Myofibroblast differentiation depends on environmental cues, including tension in the matrix and a variety of soluble mediators, such as growth factors, steroid hormones, cytokines and chemokines (Tomasek *et al.*, 2002; Gabbiani, 2003; Wipff *et al.*, 2007; Wynn, 2007, 2008; Luo *et al.*, 2014; Feng *et al.*, 2016). The central player of myofibroblast differentiation is TGF-β. It is induced by mechanical tension, induces α-SMA expression (Desmoulière *et al.*, 1993), and enhances the synthesis of both collagens (Lindahl *et al.*, 2002) and ED-A fibronectin (Serini *et al.*, 1998). The cross-linking of the ECM may further activate resident cells to myofibroblastic transition (Desmouliere *et al.*, 2005; Ho *et al.*, 2014). In addition to TGF-β, activin-A has been demonstrated to play an important role in tissue repair as well as fibrosis (Werner *et al.*, 1999). Both TGF-β and activin-A are highly expressed in uterine leiomyoma (Dou *et al.*, 1996; Tang *et al.*, 1997; Ciarmela *et al.*, 2011a) where their abilities to enhance synthesis of ECM components, such as collagen 1A1, CTGF, fibronectin, versican and PAI-1 (Arici and Sozen, 2000; Ding *et al.*, 2004b; Joseph *et al.*, 2010; Islam *et al.*, 2014a), supports their roles in driving myofibroblast differentiation. Recently, Feng *et al.* reported that after a biological insult of serum starvation and serum add-back, myometrial cells undergo a transition to a myofibroblast-like phenotype that was related to an increased activation of TGF-β signaling via the Smad 2/3 pathway (Feng *et al.*, 2016). These authors further showed that in response to TGF-β3 treatment during the biological insult, myometrial cells migrated into nodules containing collagen and fibronectin (Feng *et al.*, 2016). Using transmission electron microscopy, they also found myofibroblast-like cells and fibril-like structures in the extracellular spaces of the nodules (Feng *et al.*, 2016).

In addition to TGF-β and activin-A, TNF-α can be considered as an important regulator of myofibroblast differentiation. Indeed, TNF-α increases the expression of activin-A in both myometrial and leiomyoma cells (Islam *et al.*, 2013b) and thereby promotes the fibrotic role of activin-A in the process of myofibroblast differentiation following fibrosis.

Estrogen and progesterone have been reported to increase the proliferation of both leiomyoma and smooth muscle fibroblasts compared to untreated groups (Luo *et al.*, 2014). Estrogen-mediated fibroblast proliferation was mediated, at least in part, by increased

Table II Current and potential treatments for leiomyoma that target the extracellular matrix.

Treatment modalities	Fibrosis-related molecular targets	Effects on leiomyoma and related symptoms	Safety and side effects
Current treatments			
Leuprolide acetate	↓TGF-β1, ↓TGF-β3, ↓TGF-βR type I, ↓TGF-βR type II, ↓Smad4, ↓pSmad3, ↑Smad7, ↓ collagen type I, ↓↑fibronectin, ↓versican, ↓fibromodulin, ↓TIMP-1, ↑MMP-1, ↑MMP-2, ↑MMP-3, and ↑MMP-9	(I) Reduces uterine and leiomyoma volume (Stovall *et al.*, 1995) (II) Alleviates bleeding and increase hemoglobin levels (Stovall *et al.*, 1991)	(I) Prolonged therapy (>6 months) is not recommended (II) Side effects-hot flashes, vaginitis and bone loss (Stovall *et al.*, 1995)
Cetrorelix acetate	↓COL1A1, ↓fibronectin, and ↓versican variant V0	(I) Decreases uterine and leiomyoma volume (Gonzalez-Barcena *et al.*, 1997; Felberbaum *et al.*, 2001; Engel *et al.*, 2007) (II) Abolishes symptoms of menorrhagia and reduce uterine pain (Engel *et al.*, 2007)	(I) Side effects-amenorrhea and hot flashes (Gonzalez-Barcena *et al.*, 1997)
Asoprisnil	↓TGF-β3, ↓TGF-βR type II, ↑EMMPRIN, ↑MMP-1, ↑MT1-MMP, ↓TIMP-1, ↓TIMP-2, ↓ collagen type I and ↓collagen type III	(I) Suppress leiomyoma and uterine volume (Chwalisz *et al.*, 2007) (II) Reduces uterine bleeding as well as bloating and pelvic pressure (Chwalisz *et al.*, 2007)	(I) Abnormal endometrial changes (Williams *et al.*, 2007) (II) Side effects-hot flashes or night sweats (Chwalisz *et al.*, 2007)
Ulipristal acetate	↑MMP-1, ↑MMP-2, ↑MMP-3, ↑MMP-8, ↑MMP-9, ↑EMMPRIN, ↓TIMP-1, ↓TIMP-2, ↓ collagen type I, ↓collagen type III and ↓fibronectin	(I) Reduces leiomyoma and uterine volume (Donnez *et al.*, 2012a, 2012b., 2015) (II) Controls bleeding and pain (Donnez *et al.*, 2015) (III) Improves quality of life (Donnez *et al.*, 2012a, 2012b., 2015)	(I) Quite well tolerated (Donnez *et al.*, 2015). (II) Side effects-hot flashes and headaches (Donnez *et al.*, 2015)
Mifepristone	↓COL1A1, ↓fibronectin, ↓versican and ↓dermatopontin	(I) Reduces uterine and leiomyoma volume (Esteve *et al.*, 2012) (II) Alleviates hypermenorrhea, menstrual blood loss, pelvic painandpressure, anemia and dysmenorrhea (Shen *et al.*, 2013; Yerushalmi *et al.*, 2014) (III) Reduces leiomyoma cell viability and proliferation (Chung *et al.*, 2014)	(I) No endometrial hyperplasia or cellular atypia(Yerushalmi *et al.*, 2014) (II) Side effects-hot flashes, nausea, weakness, abdominal pain and vaginal discharge (Yerushalmi *et al.*, 2014)
Raloxifene	↓Collagen and ↓MMP-2	(I) prevents progression of uterine leiomyoma (Jirecek *et al.*, 2004)	(I) quite well tolerated (Jirecek *et al.*, 2004) (II) side effects-hot flashes and leg cramps (Martino *et al.*, 2005), and risk of venous thromboembolism (Ettinger *et al.*, 1999)
Investigational compounds			
CP8947	↓COL1A1 and ↓COL7A1	(I) Inhibits the leiomyoma cell proliferation (Catherino *et al.*, 2010) (II) No effect on myometrial cell proliferation (Catherino *et al.*, 2010)	Limited
2-methoxyestradiol	↓Collagen type I, ↓Collagen type III, ↓PAI-1, ↓CTGF, ↓α-SMA, ↓pSmad2/3 and ↓PI3K/Akt/mTOR	(I) Inhibits cell proliferation in rat and human leiomyoma cells (Salama *et al.*, 2006) (II) induces apoptosis in rat and human leiomyoma cells(Salama *et al.*, 2006)	(I) Well tolerated (Harrison *et al.*, 2011) (II) Side effects-hot flashes, muscle cramps, headache, fatigue or weakness, nausea, vomiting, diarrhea, gastrointestinal hemorrhage and hyponatremia (Rajkumar *et al.*, 2007)
Liarozole	↓COL1A1, ↓COL4A2, ↓versican, ↓fibromodulin, ↓fibronectin and ↓TGF-β3	(I) Inhibits proliferation of both myometrial and leiomyoma cells (Gilden *et al.*, 2012)	(I) Well tolerated (Denis *et al.*, 1998) (II) Side effects-skin disorders and dryness of mouth/eyes/lips (Goss *et al.*, 2000)
All-trans retinoic acid	↓Collagen 1, ↓collagen 4, ↓fibronectin, ↓versican and ↓TGF-β3	(I) Downregulates immortalized leiomyoma cell proliferation (Malik *et al.*, 2008)	(I) Quite well tolerated (Kurzrock *et al.*, 1993; Böcher *et al.*, 2008) (II) Side effects-transient headache, dry skin and mucosa, nausea and vomiting, myalgias or muscle pain, dyspnea (shortness of breath), and sensorineural hearing loss (Kurzrock *et al.*, 1993; Böcher *et al.*, 2008)

Continued

Table II *Continued*

Treatment modalities	Fibrosis-related molecular targets	Effects on leiomyoma and related symptoms	Safety and side effects
Vitamin D	↓fibronectin, ↓collagen type I, ↓PAI-1, ↓pSmad2, ↓Wnt4, ↓β-catenin, ↓mTOR, ↓fibromodulin, ↓biglycan and ↓versican	(I) Shrinks uterine leiomyoma tumors in Eker rat model (Halder *et al.*, 2012)	Limited
Celecoxib	↓collagen A, ↓fibronectin, ↓PDGF, and ↓TGF-β	(I) Inhibits leiomyoma cell proliferation (Ke *et al.*, 2013) (II) No effect on myometrial cell proliferation (Ke *et al.*, 2013)	(I) Increases the risk of myocardial infarction, stroke or heart failure (Solomon *et al.*, 2005)
Tranilast	↓COL1A1, ↓fibronectin ↓versican and ↓activin-A	(I) Inhibits proliferation of myometrial and leiomyoma cells (Shime *et al.*, 2002; Islam *et al.*, 2012)	(I) Well tolerated with low toxicity (Konneh, 1998)
Pirfenidone	↓collagen type I, ↓collagen type III	(I) Inhibits proliferation of myometrial and leiomyoma cells (Lee *et al.*, 1998)	(I) Rather well tolerated by patients with idiopathic pulmonary fibrosis (Chaudhuri *et al.*, 2014) (II) Gastrointestinal adverse effects (Chaudhuri *et al.*, 2014)
Halofuginone	↓collagen type I (a1), ↓collagen type III (a1) and ↓TGF-β1	(I) Reduces uterine leiomyoma volume in a mouse xenograft model (Koohestani *et al.*, 2016) (II) Inhibits both myometrial and leiomyoma cell proliferation (Grudzien *et al.*, 2010) (III) Induces apoptosis (Grudzien *et al.*, 2010)	(I) Appears to be safe and well tolerated (De Jonge *et al.*, 2006) (II) No clinically adverse events (De Jonge *et al.*, 2006)
Curcumin	↓fibronectin	(I) Inhibits leiomyoma cell proliferation (Malik *et al.*, 2009)	(I) Appears to be safe and well tolerated by patients (Gupta *et al.*, 2013) (II) Side effects-nausea, diarrhea, headache, rash and yellow stool (Gupta *et al.*, 2013)
Resveratrol	↓fibronectin, ↓collagen types I, ↓collagen III, ↓fibromodulin. ↓biglycan, and ↓MMP-9, ↑TIMP2	(I) Inhibits proliferation of human uterine leiomyoma cells (Catherino *et al.*, 2011)	(I) Appears safe and well tolerated by patients (Turner *et al.*, 2015) (II) Adverse events-nausea, diarrhea and weight loss (Turner *et al.*, 2015)
Collagenase *C. histolyticum*	↓ collagen	(I) Reduces fibrosis (collagen) and tissue stiffness in uterine leiomyoma (Jayes *et al.*, 2016)	Limited

secretion of TGF-β and IGF-1 as well as activation of MEK/ERK1/2 and AKT pathways (Luo *et al.*, 2014). The effect of estrogen on fibroblast activation was documented by the observation that expression of FAP as well as collagen I, fibronectin and laminin proteins was increased in estrogen-treated leiomyoma and smooth muscle fibroblasts compared to untreated controls (Luo *et al.*, 2014). FAP knockdown was reported to attenuate the estrogen-mediated proliferation of fibroblasts, as well as phosphorylation levels of MEK, ERK, AKT and protein levels of c-fos (Luo *et al.*, 2014). The decreased protein expression of ECM components (collagen I, fibronectin and laminin) was also observed in leiomyoma fibroblasts compared to untreated controls in response to suppression of FAP expression (Luo *et al.*, 2014). These results suggest that the effect of estrogen on fibroblast cell activation is mediated partially through the FAP pathway.

Inflammation is a key component of the wound healing process. However, local chronic inflammation makes a suitable microenvironment for development of fibrosis. Chronic inflammation is characterized by infiltration of mononuclear immune cells (macrophages, monocytes, lymphocytes and plasma cells). Of note, inflammatory cells, in particular macrophages, are widely accepted as important regulators of cytokines and growth factors during the wound healing process (Leibovich and Ross, 1975). Recently, we found higher numbers of macrophages present inside and in the vicinity of leiomyoma compared to the more distant surrounding myometrium (Protic *et al.*, 2016).

Chronic inflammatory reactions are induced by a variety of stimuli, including chemical insults, radiation, persistent infections, tissue injury, autoimmune reactions and allergic responses (Wynn, 2008). In the uterus, reproductive events, including ovulation, menstruation and implantation, may trigger inflammatory reactions. In addition, many factors have been hypothesized to increase inflammation in uterus, such as infection, injury, talc use, an intrauterine device, cesarean section, male reproductive proteins and stress (work, home or perceived racism) (Wegienka, 2012). Repeated stimulation by reproductive events, mechanical forces, injury, hypoxia and oxidative stress may also create a chronic inflammatory state in the uterus (Wegienka, 2012; Fletcher *et al.*, 2013; Leppert *et al.*, 2013; Santulli *et al.*, 2013). Regardless of the cause of chronic uterine inflammation, it is under this condition that

myofibroblasts cells produce ECM in an unregulated manner and fail to undergo normal apoptosis leading to fibrosis.

Although most women experience causes of uterine inflammation, e.g. reproductive events, they all do not have leiomyoma. This observation suggests that initiation of uterine leiomyoma does not solely depend on inflammation. There are other factors that may influence the risk of developing leiomyoma under the chronic inflammatory condition. Accumulated evidence suggests that genetic and epigenetic factors may influence the risk for developing leiomyoma (Gallagher and Morton, 2016; Yang et al., 2016). In addition, black race, heredity, nulliparity, obesity, polycystic ovary syndrome, diabetes and hypertension are associated with increased risk of this tumor (Okolo, 2008).

Extracellular matrix as a therapeutic target of current and future medical treatments

Several classes of compounds including GnRH agonizts, GnRH antagonists, selective progesterone receptor modulators, antiprogestin and natural compounds have been studied as medical treatments that target ECM in uterine leiomyoma (Table II).

Current treatments

Leuprolide acetate

Leuprolide acetate is a synthetic analog of GnRH. GnRH is produced in the hypothalamus of the brain and after its release travels to the pituitary gland where it stimulates the production of LH and follicle stimulating hormone (FSH). Through peripheral circulation, LH and FSH travel to the ovaries where they stimulate the production of estrogen. Estrogen maintains its own levels within an appropriate range by acting as a negative feedback regulator of GnRH, LH and FSH production. Leuprolide acetate acts by producing an initial stimulation of FSH and LH as well as estrogen but after a few weeks, levels of LH and FSH drop because the pituitary gland stops responding to GnRH and leuprolide. This induces a state of hypoestrogenism, which has been used for the treatment of leiomyoma (Stovall et al., 1991). Leuprolide acetate is able to alleviate bleeding and increase hemoglobin levels (Stovall et al., 1991) as well as reduce uterine and leiomyoma volume (Stovall et al., 1995). However, this treatment is associated with some side effect related to hypoestrogenism, such as hot flashes, vaginitis and bone loss, which constitute the major limitation of long-term use (Stovall et al., 1995). The therapeutic effects of leuprolide acetate on leiomyoma are mediated by regulation of TGF-β receptor signaling and substantial tissue remodeling (Dou et al., 1997; Chegini et al., 2003; Ding et al., 2004b). Leuprolide acetate was reported to inhibit TGF-β1, TGF-β3, TGF-βR type I and type II as well as Smad4 and pSmad3 levels while increasing Smad7 expression in both myometrium and leiomyoma relative to untreated controls (Dou et al., 1996; Chegini et al., 2003). Leuprolide acetate also inhibited fibronectin mRNA expression in myometrial cells with a moderate increase in its expression in leiomyoma cells while also inhibiting type I collagen expression in both myometrial and leiomyoma cells (Ding et al., 2004b). A recent study reported that 3D leiomyoma cultures exposed to estrogen and progesterone demonstrated an increased expression of collagen-1, fibronectin and versican and this effect was inhibited by leuprolide acetate (Malik et al., 2016). TGF-β1 increased the expression of fibromodulin in myometrial cells, whereas leuprolide acetate inhibited this effect in both myometrial and leiomyoma cells (Levens et al., 2005). Dou et al. reported that leuprolide acetate treatment also induced a significant decrease in TIMP-1, and an increase in MMP-1, MMP-2, MMP-3 and MMP-9 mRNA expression in both leiomyoma and myometrium compared with untreated groups (Dou et al., 1997).

Cetrorelix acetate

Cetrorelix acetate, a GnRH antagonist, has been reported to decrease mean uterine and leiomyoma volume in pre-menopausal women (Gonzalez-Barcena et al., 1997; Felberbaum et al., 2001; Engel et al., 2007). This compound can abolish symptoms of menorrhagia and reduce uterine pain (Engel et al., 2007). However, cetrorelix acetate is associated with amenorrhea and hot flashes (Gonzalez-Barcena et al., 1997) and its therapeutic use is also limited by its prohibitive cost and requirement for daily injections (Kashani et al., 2016). Britten et al. investigated the effect of cetrorelix acetate on ECM production in human uterine leiomyoma and patient-matched myometrial cells (Britten et al., 2012). They found that cetrorelix decreased mRNA and protein expression of COL1A1, fibronectin and versican variant V0 in a time dependent manner in leiomyoma cells compared to untreated cells (Britten et al., 2012). In 3D leiomyoma cultures, cetrorelix also decreased estrogen- and progesterone-induced expression of collagen-1, fibronectin and versican (Malik et al., 2016).

Asoprisnil

Asoprisnil is a selective progesterone receptor modulator that shows a high degree of receptor and tissue selectivity. It has high-binding affinity for progesterone receptors, no binding affinity for estrogen or mineralocorticoid receptors, moderate affinity for glucocorticoid receptors and low affinity for androgen receptors. Asoprisnil has been reported to suppress leiomyoma and uterine volume as well as uterine bleeding and was associated with significant reduction in bloating and pelvic pressure (Chwalisz et al., 2007). The safety profile indicates that asoprisnil treatment is associated with endometrial changes including abnormal vascular growth (Williams et al., 2007). The adverse events, such as hot flashes or night sweats, were also detected but they were mild or moderate in severity, because asoprisnil does not induce a hypoestrogenic state (Chwalisz et al., 2007; Kashani et al., 2016). Asoprisnil exerts therapeutic effects on leiomyoma by regulating growth factor expression as well as ECM turnover and tissue remodeling (Wang et al., 2006; Morikawa et al., 2008). Treatment of leiomyoma cells with asoprisnil caused a decrease in the levels of TGF-β3 mRNA and protein and phosphorylated TGF-βR type II receptor compared to untreated controls (Wang et al., 2006). Morikawa and co-investigators reported that asoprisnil treatment also significantly increased levels of EMMPRIN, MMP-1 and MT1-MMP (membrane type 1-MMP) while decreasing levels of TIMP-1, TIMP-2, collagen type I and type III in cultured leiomyoma cells compared to untreated control cells (Morikawa et al., 2008). This group also reported that asoprisnil had no effect on protein contents of ECM and ECM-remodeling enzymes in myometrial cells (Morikawa et al., 2008).

Ulipristal acetate

Ulipristal acetate (also known as CDB-2914) is a selective progesterone receptor modulator that binds to progesterone receptors A and B with high affinity. Use of this drug has been approved in Europe and Canada for preoperative leiomyoma treatment (Melis *et al.*, 2012). Ulipristal acetate is able to reduce leiomyoma and uterine volume and improves leiomyoma-related symptoms such as bleeding and pain as well as quality of life (Donnez *et al.*, 2012a, 2012b, 2015). While this treatment is associated with some adverse events, such as hot flashes and headaches, and breast tenderness, these occurred in ≤10% of patients as circulating estradiol levels are maintained in the mid-follicular range throughout the treatment duration (Kashani *et al.*, 2016). Moreover, it may be associated with physiologic endometrial changes (PAECs) that consist of benign cystic glandular dilation but these have not been associated to date with an increased risk of endometrial hyperplasia or malignancy (Donnez *et al.*, 2015). Ulipristal acetate exerts therapeutic effects on leiomyoma growth by regulating the fibrotic process and its use results in lower ECM volume and higher MMP-2 expression in women with symptomatic leiomyoma compared to untreated leiomyoma (Courtoy *et al.*, 2015). In cultured leiomyoma cells, ulipristal acetate significantly increased EMMPRIN, MMP-1 and MMP-8 protein contents as well as MMP-1, MMP-2, MMP-3 and MMP-9 mRNA levels, while decreasing mRNA and protein levels of TIMP-1 and TIMP-2 as well as collagen types I and III content, without comparable effects on cultured normal myometrial cells (Xu *et al.*, 2008). Recently, we also demonstrated that ulipristal acetate can block activin-A induction of fibronectin mRNA expression in myometrial and leiomyoma cultured cells (Ciarmela *et al.*, 2014).

Mifepristone

Mifepristone (also known as RU-486, mifegyne, mifeprex) is a synthetic steroid with antiprogesterone and antiglucocorticoid activity. It binds progesterone receptors and competitively antagonizes progesterone binding and signaling. Several clinical studies have demonstrated that mifepristone can reduce uterine and leiomyoma volume as well as alleviate leiomyoma-related symptoms including hypermenorrhea, menstrual blood loss, pelvic pain or pressure, anemia and dysmenorrhea (Esteve *et al.*, 2012, 2013; Shen *et al.*, 2013; Yerushalmi *et al.*, 2014). A recent study reported that no endometrial hyperplasia or cellular atypia was observed after vaginal mifepristone treatment (Yerushalmi *et al.*, 2014). However, some side effects, including hot flashes (10.3%), nausea (6.9%), weakness (6.9%), abdominal pain (24.1%) and vaginal discharge (20.7%), were observed at some point during the course of the 3 month study (Yerushalmi *et al.*, 2014). Moreover, the increased risk of developing endometrial hyperplasia (28%) (10/36) during treatment with mifepristone raised safety concerns about its use in the management of leiomyoma (Steinauer *et al.*, 2004; Guo and Segars, 2012). Antifibrotic effects of mifepristone have recently been demonstrated by Patel *et al.* (2016). This group reported that the progesterone agonist R5020 directly stimulated production of several ECM components including COL1A1, fibronectin, versican and dermatopontin in human leiomyoma cells compared to untreated cells and this effect was inhibited by mifepristone (Patel *et al.*, 2016). Additionally, mifepristone can significantly reduce leiomyoma cell viability and proliferation (Chung *et al.*, 2014) at least partly by downregulation of LAT2 (L-type amino acid transporter 2) mRNA expression (Luo *et al.*, 2009). Furthermore, Yin *et al.* reported that mifepristone robustly up-regulated mRNA and protein levels of the known tumor suppressor KLF11 in leiomyoma smooth muscle cells (Yin *et al.*, 2010).

Raloxifene

Raloxifene is a selective estrogen receptor modulator that interacts with estrogen receptors to elicit tissue-specific responses. It inhibits the growth of uterine leiomyoma in pre-menopausal women (Jirecek *et al.*, 2004). Raloxifene is quite well tolerated (Jirecek *et al.*, 2004) but has been associated with the risk of venous thromboembolism (Ettinger *et al.*, 1999) as well as hot flashes and leg cramps (Martino *et al.*, 2005). Raloxifene appears to inhibit collagen biosynthesis in leiomyoma cells while only slightly affecting collagen biosynthesis in control myometrial cells (Zbucka *et al.*, 2008). Raloxifene was also found to inhibit MMP-2 in leiomyoma as well as in control myometrial cells (Zbucka *et al.*, 2008).

Investigational compounds

CP8947

CP8947 is a novel non-steroidal selective progesterone receptor modulator derived from *Penicillium oblatum*. It has high selectivity for progesterone receptors and lacks affinity for estrogen receptor-α, androgen receptor and glucocorticoid receptor. Catherino *et al.* investigated the effect of CP8947 on cell proliferation and ECM components in leiomyoma cells (Catherino *et al.*, 2010). They found that CP8947 was effective in inhibiting leiomyoma cell proliferation without disrupting myometrial cell proliferation and that it also decreased mRNA expression of COL1A1 and COL7A1 (Catherino *et al.*, 2010).

2-Methoxyestradiol

2-Methoxyestradiol is a naturally occurring estradiol metabolite with low affinity for estrogen receptors. 2-Methoxyestradiol is currently being evaluated in ongoing advanced phases of clinical trials in patients with multiple myeloma, glioblastoma, ovarian cancer, metastatic renal cell carcinoma and prostate cancer. It is well tolerated by patients but associated with some adverse events such as hot flashes, muscle cramps, headache, fatigue or weakness, nausea, vomiting, diarrhea, gastrointestinal hemorrhage and hyponatremia (Rajkumar *et al.*, 2007; Harrison *et al.*, 2011). 2-Methoxyestradiol has been reported to induce apoptosis as well as inhibit cell proliferation and collagen production in rat and human leiomyoma cells (Salama *et al.*, 2006). Salama *et al.* investigated the antifibrotic effect of 2-methoxyestradiol on TGF-β3 mediated fibrosis-related factors in leiomyoma cells (Salama *et al.*, 2012). They found that 2-methoxyestradiol abrogated TGF-β3-induced expression of collagen types I and III, PAI-1, CTGF and α-SMA in immortalized human uterine leiomyoma smooth muscle cells compared to untreated controls (Salama *et al.*, 2012). 2-Methoxyestradiol also inhibited TGF-β3-induced activation of the PI3K/AKT/mTOR pathway as well as ameliorated TGF-β3-induced phosphorylation and nuclear translocation of Smad2/3 in this cell type (Salama *et al.*, 2012). It has been shown that relatively high concentrations of 2-methoxyestradiol can also induce spindle aberrations in oocytes (Eichenlaub-Ritter *et al.*, 2007).

Liarozole

Liarozole inhibits the cytochrome P450 (CYP)-dependent catabolism of retinoic acid and thereby increases intracellular retinoic acid levels. Liarozole is well tolerated in clinical studies and has been studied as treatment for prostate cancer (Denis *et al.*, 1998) and breast cancer (Goss *et al.*, 2000). The adverse events were predominantly dermatological including skin disorders (88%) and dryness of mouth/eyes/lips (69%) (Goss *et al.*, 2000). The antifibrotic effects of liarozole have been studied in leiomyoma cells (Gilden *et al.*, 2012; Levy *et al.*, 2014). Gilden and co-investigators reported that liarozole can inhibit proliferation of both myometrial and leiomyoma cells at supraphar-macologic concentrations and also decrease mRNA and protein expression of COL1A1, COL4A2, versican, fibromodulin and fibronectin in a dose-dependent manner in leiomyoma cells compared with myometrial cells (Gilden *et al.*, 2012). Interestingly, they found no statistically significant alteration in ECM regulation in liarozole treated myometrial cells (Gilden *et al.*, 2012). A recent study reported that liarozole can inhibit TGF-β3 and TGF-β3 induction of ECM components, including versican, COL1A1 and fibronectin in human three-dimensional leiomyoma cultures (Levy *et al.*, 2014).

All-trans retinoic acid

All-trans retinoic acid (also known as tretinoin) is a derivative of vitamin A that functions as a ligand for the retinoic acid receptor (RAR). It is quite well tolerated by patients but has been associated with common side effects including transient headache, dry skin and mucosa, nausea and vomiting, myalgias or muscle pain, dyspnea (shortness of breath) and sensorineural hearing loss (Kurzrock *et al.*, 1993; Böcher *et al.*, 2008). Human uterine smooth muscle cells express retinoic acid receptors RAR α, β and γ and retinoid X receptors RXR α and β as well as all-trans retinoic acid (Boettger-Tong *et al.*, 1997). Treatment of immortalized leiomyoma cells with all-trans retinoic acid was reported to downregulate cell proliferation as well as protein production of ECM components including collagen 1, collagen 4, fibronectin and versican as well as mRNA expression of TGF-β3 compared to untreated controls (Malik *et al.*, 2008).

Vitamin D

Vitamin D is a fat-soluble vitamin found in many foods including fish, eggs, fortified milk and cod liver oil. Its major physiologically relevant forms are D2 (ergocalciferol) and D3 (cholecalciferol). Several recent *in vitro* and *in vivo* studies implicate vitamin D insufficiency as an important contributor to the development of uterine leiomyoma (Halder *et al.*, 2012; Baird *et al.*, 2013; Paffoni *et al.*, 2013). Women with sufficient vitamin D have been reported to have an estimated 32% lower odds of leiomyoma compared to women with vitamin D insufficiency (Baird *et al.*, 2013). Similarly, Paffoniand *et al.* reported that women with a vitamin D deficiency experienced 2.4 times more leiomyoma compared to women with an adequate level of vitamin D (Paffoni *et al.*, 2013). Using the Eker rat model, Halder and co-investigators found that vitamin D3 [1,25(OH)2D3] treatment was able to shrink uterine leiomyoma tumors (Halder *et al.*, 2012). The therapeutic effect of vitamin D3 on leiomyoma was mediated, at least in part, by regulation of TGF-β responsive genes (Halder *et al.*, 2011, 2013), as well as Wnt/β-catenin and mTOR signaling pathways (Al-Hendy *et al.*, 2016). Vitamin D3 reduced TGF-β3-induced expression of fibronectin, collagen type I and PAI-1 protein in human uterine leiomyoma cells (Halder *et al.*, 2011). Vitamin D3 also reduced TGF-β3-induced Smad2 phosphorylation as well as Smad2 and Smad3 nuclear translocation in human uterine leiomyoma cells (Halder *et al.*, 2011). Al-Hendy *et al.* reported that vitamin D3 can reduce the levels of Wnt4 and β-catenin as well as mTOR in both immortalized and primary human uterine leiomyoma cells (Al-Hendy *et al.*, 2016). Furthermore, vitamin D3 was reported to reduce mRNA and protein levels of fibromodulin, biglycan and versican in human uterine leiomyoma cells (Halder *et al.*, 2013). The ability of vitamin D3 to downregulate ECM (fibronectin and collagen type 1) expression as well as Wnt4/β-catenin and cell proliferation was further confirmed by the observation that silencing expression of the vitamin D receptor (VDR) gene in normal myometrial cells increased ECM production as well as Wnt4/β-catenin and cell proliferation (Al-Hendy *et al.*, 2016). Initial results regarding the opportunity for Vitamin D3 supplementation in women with leiomyoma are encouraging, but they must be confirmed by further studies (Ciavattini *et al.*, 2016).

Celecoxib

Celecoxib is an inhibitor of cyclooxygenase 2 (COX-2) that is commonly used to manage pain or inflammation. The function of COX-2 is to convert arachidonic acid into prostaglandin H2, a common substrate for specific prostaglandin synthases. Uterine leiomyoma cells have higher levels of COX-2 mRNA and protein than myometrial cells (Ke *et al.*, 2013). A recent study reported that celecoxib can significantly inhibit uterine leiomyoma cell proliferation without affecting proliferation of healthy myometrial smooth muscle cells (Ke *et al.*, 2013). Celcoxib can also reduce expression of collagen A, fibronectin, PDGF and TGF-β at the mRNA level in uterine leiomyoma cells compared to untreated controls (Park *et al.*, 2014). Although initial results are encouraging, the potential health risks may hinder the use of celecoxib for the treatment uterine leiomyoma. Clinical evidence indicates that celecoxib use is associated with increased risk of cardiovascular events, myocardial infarction, stroke and heart failure (Solomon *et al.*, 2005).

Tranilast

Tranilast is an orally administered synthetic drug of low toxicity used for the treatment of allergic disorders in Japan and South Korea. It is well tolerated by patients (Konneh, 1998) supporting the possibility for its use to treat uterine leiomyoma. The antifibrotic effects of tranilast have been reported in myometrial and leiomyoma cells (Islam *et al.*, 2014b). Recently, we demonstrated that tranilast can decrease expression of COL1A1 and fibronectin at mRNA and protein levels, as well as versican and activin-A at mRNA levels in myometrial and leiomyoma cells (Islam *et al.*, 2014b). Additionally, tranilast can also inhibit the proliferation of uterine myometrial and leiomyoma cells (Shime *et al.*, 2002; Islam *et al.*, 2012).

Pirfenidone

Pirfenidone is a synthetic pyridone compound used for the treatment of idiopathic pulmonary fibrosis. It is rather well tolerated by patients, with adverse effects that are predominantly gastrointestinal (Chaudhuri *et al.*, 2014). The antifibrotic potential of pirfenidone has been studied in leiomyoma cells by Lee *et al.* (1998). They reported

that pirfenidone was effective in regulating proliferation of myometrial and leiomyoma cells in vitro as well as in reducing the mRNA level of collagen types I and III in a dose-dependent manner (Lee *et al.*, 1998).

Halofuginone

Halofuginone, an analog of febrifugine, is a small alkaloid isolated from the plant *Dichroa febrifuga*. It has been used as a coccidiostat (an antiprotozoal agent) in chickens since the 1960s. Phase I/II clinical trials of halofuginone have been completed in patients with progressive advanced solid tumors and HIV-related Kaposi's sarcoma. Halofuginone at dose 0.5 mg/d appears to be safe and well tolerated, with no clinically adverse events (De Jonge *et al.*, 2006). The antifibrotic effect of halofuginone on uterine leiomyoma has been reported (Grudzien *et al.*, 2010). Grudzien *et al.* reported that halofuginone can significantly reduce collagen type I (a1), collagen type III (a1) and TGF-β1 mRNA levels in leiomyoma and myometrial cells compared to corresponding untreated cells (Grudzien *et al.*, 2010). Halofuginone can also inhibit both myometrial and leiomyoma cell proliferation by inducing apoptosis and inhibiting DNA synthesis in a dose-dependent manner (Grudzien *et al.*, 2010). A recent study by Koohestani *et al.* reported that halofuginone was effective in reducing uterine leiomyoma volume in a mouse xenograft model (Koohestani *et al.*, 2016). This group found a 35–40% reduction in leiomyoma/tumor volume in mice carrying human uterine leiomyoma xenografts after treatment with halofuginone compared to control groups. The halofuginone-induced reduction of tumor volume was accompanied by decreased cell proliferation and increased apoptosis (Koohestani *et al.*, 2016). However, these authors found no significant difference in the content of collagen between helofuginone-treated mice carrying human uterine leiomyoma xenografts and control groups (Koohestani *et al.*, 2016).

Curcumin

Curcumin is a polyphenol derived from rhizome of turmeric (*Curcuma longa*). Turmeric is commonly used in Asian foods. It has shown beneficial effects in a plethora of human diseases. Extensive clinical trials indicate that curcumin is safe and well tolerated by patients. However, this treatment is associated with some undesired adverse effects such as nausea, diarrhea, headache, rash and yellow stool (Gupta *et al.*, 2013). Antifibrotic effects of curcumin on human uterine leiomyoma cells have been demonstrated by Malik *et al.* (2009). This group reported that curcumin can inhibit mRNA expression of fibronectin in leiomyoma cells compared to untreated controls (Malik *et al.*, 2009). They also found that curcumin can inhibit proliferation of leiomyoma cells without affecting patient-matched myometrial cells (Malik *et al.*, 2009). These effects were mediated by inducing caspase-3 and caspase-9 protein expression as well as inhibiting ERK 1/2 and NF-κB protein expression (Malik *et al.*, 2009).

Resveratrol

Resveratrol is a polyphenolic compound found in peanuts, grapes and some berries and is produced by plants in response to environmental stress, pathogen infection and ultraviolet radiation. Resveratrol is safe and well tolerated by patients, with common adverse events including nausea, diarrhea and weight loss (Turner *et al.*, 2015). The ability of resveratrol to interfere with ECM formation and deposition in multiple diseases has recently been discussed by Agarwal and Agarwal (2017). Resveratrol appears to reduce expression of fibronectin, collagen types I and III as well as fibromodulin and biglycan at mRNA and/or protein levels in ELT-3 cells and/or human uterine leiomyoma smooth muscle cells compared to corresponding untreated cells (Catherino *et al.*, 2011; Wu *et al.*, 2016). Resveratrol also reduced MMP-9 protein expression while increasing TIMP2 protein expression in ELT-3 cells and healthy uterine smooth muscle cells compared to corresponding untreated cells (Wu *et al.*, 2016). Furthermore, resveratrol was reported to inhibit proliferation and induce apoptosis and cell cycle arrest in human uterine leiomyoma cells compared to untreated controls (Catherino *et al.*, 2011).

Collagenase C. histolyticum

Collagenase *C. histolyticum* (CCH) is a bacterial enzyme that breaks down collagen. CCH has been approved by the US FDA for the treatment of Dupuytren's contracture (a thickening of the fibrous tissue layer underneath the skin of the palm and fingers) and Peyronie's disease (a connective tissue disorder of the penis). A recent study by Jayes *et al.* investigated whether CCH was effective in the digestion of interstitial collagen in uterine leiomyoma (Jayes *et al.*, 2016). They found 37–77% fibrosis in untreated leiomyoma, indicating the collagen-rich nature of these tumors (Jayes *et al.*, 2016). A reduced amount of fibrosis ranging from 5.3 to 2.4% was recorded after treatment with CCH. Furthermore, complete digestion of collagen fibrils was confirmed by transmission electron microscopy. Tissue stiffness was also reduced with CCH treatment (Jayes *et al.*, 2016). The above results suggest that CCH may reduce leiomyoma size and bulk symptoms possibly through collagen digestion and by modulating mechanotransduction process. Clinical trials are necessary to evaluate the safety and efficacy of CCH.

Conclusions and future perspectives

Uterine leiomyoma expresses a wide variety of ECM components, including collagens, fibronectin, laminins, proteoglycans and integrins as well as MMPs and TIMPs. ECM proteins can induce mechanotransduction, thus activating pleiotropic intracellular signaling cascades such as the integrin-Rho/p38 MAPK/ERK pathways.

ECM accumulation is regulated by growth factors, cytokines and steroid hormones. Among growth factors and cytokines, TGF-β, activin-A, PDGF and TNF-α are able to increase the synthesis of ECM components through activation of multiple signaling pathways (e.g. Smad 2/3 and MEK/ERK). In addition, estrogen and progesterone increase ECM production by regulating the expression and activity of growth factors (TGF-β and IGF), again leading to stimulation of several signaling pathways (MEK/ERK, AKT and PLCγ).

ECM may act as a reservoir of growth factors and protect them from degradation in the ECM microenvironment. ECM proteins and their receptors (integrins) can interact with growth factors independently or synergistically. MMPs can degrade ECM proteins and GAGs of proteoglycans, thereby inducing local release of soluble growth factors from their insoluble state.

Uterine leiomyoma is thought to be an inflammatory/fibrotic disorder, and possibly myofibroblasts are thought to play key role in the

process of fibrosis. Reproductive events (mainly menstruation), infection, mechanical stress, injury, oxidative stress or hypoxia may act as the initiator of inflammation in the uterus. In response to inflammation, myofibroblast cells produce ECM to promote necessary repair and tissue homeostasis, but deregulation of normal myofibroblast function may lead to fibrosis. The differentiation of myofibroblasts is regulated by TGF-β and activin-A and estrogen.

The above findings support the fact that ECM is a major molecular switch that can be considered to be a crucial therapeutic target to control abnormal growth of leiomyoma. Currently, several types of drugs are available for leiomyoma treatment but none of them has been introduced specifically as antifibrotic agents (Table II). Therefore, the introduction of effective antifibrotic drugs which target ECM components directly or interfere with their expression and deposition has the potential to be an effective solution for the management of uterine leiomyoma. The antifibrotic compounds can be introduced based on their ability to regulate ECM components and ECM receptors as well as growth factors, cytokines, steroid hormones and their corresponding receptors and intracellular signaling pathways, as well as miRNAs, involved in ECM production in leiomyoma. Since ECM is involved in both pathological and physiological processes, therefore, it will be an important challenge to identify compounds that specifically target unwanted fibrosis and optimize their use in interrupting the fibrotic process without disturbing the normal physiological environment.

Authors' roles

M.S.I. and P.C. conceived and designed the study. M.S.I. conducted the review of the literature and wrote the first draft of the manuscript. A.C., F.P., M.C. and P.C. supervised the work and corrected the manuscript. All authors contributed to analysis and interpretation of the data, critically revised and approved the manuscript.

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Conflict of interest

The authors have nothing to declare.

References

Abreu JG, Coffinier C, Larraín J, Oelgeschläger M, De Robertis EM. Chordin-like CR domains and the regulation of evolutionarily conserved extracellular signaling systems. *Gene* 2002;**1**:39–47.

Agarwal R, Agarwal P. Targeting extracellular matrix remodeling in disease: could resveratrol be a potential candidate? *Exp Biol Med* 2017;**4**:374–383.

Al-Hendy A, Diamond MP, Boyer TG, Halder SK. Vitamin D3 inhibits Wnt/β-catenin and mTOR signaling pathways in human uterine fibroid cells. *J Clin Endocrinol Metab* 2016;**4**:1542–1551.

Alam N, Goel HL, Zarif MJ, Butterfield JE, Perkins HM, Sansoucy BG, Sawyer TK, Languino LR. The integrin-growth factor receptor duet. *J Cell Physiol* 2007;**3**:649–653.

Albig AR, Neil JR, Schiemann WP. Fibulins 3 and 5 antagonize tumor angiogenesis in vivo. *Cancer Res* 2006;**5**:2621–2629.

Andersson-Sjöland A, Hallgren O, Rolandsson S, Weitoft M, Tykesson E, Larsson-Callerfelt A-K, Rydell-Törmänen K, Bjermer L, Malmström A, Karlsson JC. Versican in inflammation and tissue remodelling: the impact on lung disorders. *Glycobiology* 2014;**25**:243–251.

Anuar N, Di Gregoli K, Newby A, Johnson J. IL-3 induced MMP-3 expression directs angiogensis and neovascularisation in atherosclerosis. *Atherosclerosis* 2016;**244**:e4–e5.

Arici A, Sozen I. Transforming growth factor-β3 is expressed at high levels in leiomyoma where it stimulates fibronectin expression and cell proliferation. *Fertil Steril* 2000;**5**:1006–1011.

Assoian RK, Schwartz MA. Coordinate signaling by integrins and receptor tyrosine kinases in the regulation of G1 phase cell-cycle progression. *Curr Opin Genet Dev* 2001;**1**:48–53.

Baird DD, Hill MC, Schectman JM, Hollis BW. Vitamin D and the risk of uterine fibroids. *Epidemiology* 2013;**3**:447–453.

Barbarisi A, Petillo O, Di Lieto A, Melone MA, Margarucci S, Cannas M, Peluso G. 17-beta estradiol elicits an autocrine leiomyoma cell proliferation: evidence for a stimulation of protein kinase-dependent pathway. *J Cell Physiol* 2001;**3**:414–424.

Barker NM, Carrino DA, Caplan AI, Hurd WW, Liu JH, Tan H, Mesiano S. Proteoglycans in leiomyoma and normal myometrium abundance, steroid hormone control, and implications for pathophysiology. *Reprod Sci* 2015;**3**:302–309.

Benassayag C, Leroy MJ, Rigourd V, Robert B, Honore JC, Mignot TM, Vacher-Lavenu MC, Chapron C, Ferre F. Estrogen receptors (ERα/ERβ) in normal and pathological growth of the human myometrium: pregnancy and leiomyoma. *Am J Physiol* 1999;**6**:E1112–E1118.

Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat Cell Biol* 2000;**10**:737–744.

Berto AGA, Oba SM, Michelacci YM, Sampaio LO. Galactosaminoglycans from normal myometrium and leiomyoma. *Braz J Med Biol Res* 2001;**5**:633–637.

Bill HM, Knudsen B, Moores SL, Muthuswamy SK, Rao VR, Brugge JS, Miranti CK. Epidermal growth factor receptor-dependent regulation of integrin-mediated signaling and cell cycle entry in epithelial cells. *Mol Cell Biol* 2004;**19**:8586–8599.

Bissell MJ, Aggeler J. Dynamic reciprocity: how do extracellular matrix and hormones direct gene expression? *Prog Clin Biol Res* 1986;**249**:251–262.

Böcher WO, Wallasch C, Höhler T, Galle PR. All-trans retinoic acid for treatment of chronic hepatitis C. *Liver Int* 2008;**3**:347–354.

Boettger-Tong H, Shipley G, Hsu CJ, Stancel GM. Cultured human uterine smooth muscle cells are retinoid responsive. *Proc Soc Exp Biol Med* 1997;**1**:59–65.

Bogusiewicz M, Stryjecka-Zimmer M, Postawski K, Jakimiuk AJ, Rechberger T. Activity of matrix metalloproteinase-2 and-9 and contents of their tissue inhibitors in uterine leiomyoma and corresponding myometrium. *Gynecol Endocrinol* 2007;**9**:541–546.

Border WA, Okuda S, Languino LR, Ruoslahti E. Transforming growth factor-β regulates production of proteoglycans by mesangial cells. *Kidney Int* 1990;**37**:689–695.

Boulday G, Fitau J, Coupel S, Soulillou JP, Charreau B. Exogenous tissue inhibitor of metalloproteinase-1 promotes endothelial cell survival through activation of the phosphatidylinositol 3-kinase/Akt pathway. *Ann N Y Acad Sci* 2004;**1**:28–36.

Brauer R, Beck IM, Roderfeld M, Roeb E, Sedlacek R. Matrix metalloproteinase-19 inhibits growth of endothelial cells by generating angiostatin-like fragments from plasminogen. *BMC Biochem* 2011;**1**:38.

Britten JL, Malik M, Levy G, Mendoza M, Catherino WH. Gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate and GnRH antagonist cetrorelix acetate directly inhibit leiomyoma extracellular matrix production. *Fertil Steril* 2012;**5**:1299–1307.

Brown LF, Detmar M, Tognazzi K, Abu-Jawdeh G, Iruela-Arispe ML. Uterine smooth muscle cells express functional receptors (flt-1 and KDR) for vascular permeability factor/vascular endothelial growth factor. *Lab Invest* 1997;**2**:245–255.

Buttram VC Jr, Reiter RC. Uterine leiomyomata: etiology, symptomatology, and management. *Fertil Steril* 1981;**4**:433–445.

Bützow R, Fukushima D, Twardzik DR, Ruoslahti E. A 60-kD protein mediates the binding of transforming growth factor-β to cell surface and extracellular matrix proteoglycans. *J Cell Biol* 1993;**3**:721–727.

Cabrera S, Gaxiola M, Arreola JL, Ramírez R, Jara P, D'Armiento J, Richards T, Selman M, Pardo A. Overexpression of MMP9 in macrophages attenuates

pulmonary fibrosis induced by bleomycin. *Int J Biochem Cell Biol* 2007;**12**:2324–2338.

Cardozo ER, Clark AD, Banks NK, Henne MB, Stegmann BJ, Segars JH. The estimated annual cost of uterine leiomyomata in the United States. *Am J Obstet Gynecol* 2012;**3**:211. e1–211. e9.

Carrino DA, Mesiano S, Barker NM, Hurd WW, Caplan AI. Proteoglycans of uterine fibroids and keloid scars: similarity in their proteoglycan composition. *Biochem J* 2012;**2**:361–368.

Catherino WH, Malik M, Driggers P, Chappel S, Segars J, Davis J. Novel, orally active selective progesterone receptor modulator CP8947 inhibits leiomyoma cell proliferation without adversely affecting endometrium or myometrium. *J Steroid Biochem Mol Biol* 2010;**4**:279–286.

Catherino WH, Parrott E, Segars J. Proceedings from the national institute of child health and human development conference on the uterine fibroid research update workshop. *Fertil Steril* 2011;**1**:9–12.

Cavin S, Maric D, Diviani D. A-kinase anchoring protein-Lbc promotes pro-fibrotic signaling in cardiac fibroblasts. *Biochim Biophys Acta* 2014;**2**:335–345.

Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, Boulet L-P, Hamid Q. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-β, IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol* 2003;**6**:1293–1298.

Chang HL, Senaratne TN, Zhang LH, Szotek PP, Stewart E, Dombkowski D, Preffer F, Donahoe PK, Teixeira J. Uterine leiomyomas exhibit fewer stem/progenitor cell characteristics when compared with corresponding normal myometrium. *Reprod Sci* 2010;**2**:158–167.

Chaudhuri N, Duck A, Frank R, Holme J, Leonard C. Real world experiences: pirfenidone is well tolerated in patients with idiopathic pulmonary fibrosis. *Respir Med* 2014;**1**:224–226.

Chegini N, Luo X, Ding L, Ripley D. The expression of Smads and transforming growth factor beta receptors in leiomyoma and myometrium and the effect of gonadotropin releasing hormone analogue therapy. *Mol Cell Endocrinol* 2003;**1-2**:9–16.

Chegini N, Ma C, Tang XM, Williams RS. Effects of GnRH analogues, 'add-back' steroid therapy, antiestrogen and antiprogestins on leiomyoma and myometrial smooth muscle cell growth and transforming growth factor-β expression. *Mol Hum Reprod* 2002;**12**:1071–1078.

Chegini N, Tang XM, Ma C. Regulation of transforming growth factor-beta1 expression by granulocyte macrophage-colony-stimulating factor in leiomyoma and myometrial smooth muscle cells. *J Clin Endocrinol Metab* 1999;**11**:4138–4143.

Chen H-M, Lin Y-H, Cheng Y-M, Wing L-YC, Tsai S-J. Overexpression of integrin-β1 in leiomyoma promotes cell spreading and proliferation. *J Clin Endocrinol Metab* 2013;**5**:E837–E846.

Chuang T-D, Khorram O. miR-200c regulates IL8 expression by targeting IKBKB: a potential mediator of inflammation in leiomyoma pathogenesis. *PloS one* 2014;**4**:e95370.

Chuang T-D, Khorram O. Mechanisms underlying aberrant expression of miR-29c in uterine leiomyoma. *Fertil Steril* 2016;**1**:236–245. e1.

Chuang TD, Luo X, Panda H, Chegini N. miR-93/106b and their host gene, MCM7, are differentially expressed in leiomyomas and functionally target F3 and IL-8. *Mol Endocrinol* 2012a;**6**:1028–1042.

Chuang TD, Panda H, Luo X, Chegini N. miR-200c is aberrantly expressed in leiomyomas in an ethnic-dependent manner and targets ZEBs, VEGFA, TIMP2, and FBLN5. *Endocr Relat Cancer* 2012b;**4**:541–556.

Chung Y-J, Chae B, Kwak S-H, Song J-Y, Lee A-W, Jo H-H, Lew Y-O, Kim J-H, Kim M-R. Comparison of the inhibitory effect of gonadotropin releasing hormone (GnRH) agonist, selective estrogen receptor modulator (SERM), antiprogesterone on myoma cell proliferation in vitro. *Int J Med Sci* 2014;**3**:276–281.

Chwalisz K, Larsen L, Mattia-Goldberg C, Edmonds A, Elger W, Winkel CA. A randomized, controlled trial of asoprisnil, a novel selective progesterone receptor modulator, in women with uterine leiomyomata. *Fertil Steril* 2007;**6**:1399–1412.

Ciarmela P, Bloise E, Gray PC, Carrarelli P, Islam MS, De Pascalis F, Severi FM, Vale W, Castellucci M, Petraglia F. Activin-A and myostatin response and steroid regulation in human myometrium: disruption of their signalling in uterine fibroid. *J Clin Endocrinol Metab* 2011a;**03**:755–765.

Ciarmela P, Carrarelli P, Islam MS, Janjusevic M, Zupi E, Tosti C, Castellucci M, Petraglia F. Ulipristal acetate modulates the expression and functions of activin A in leiomyoma cells. *Reprod Sci* 2014;**9**:1120–1125.

Ciarmela P, Islam MS, Reis FM, Gray PC, Bloise E, Petraglia F, Vale W, Castellucci M. Growth factors and myometrium: biological effects in uterine fibroid and possible clinical implications. *Hum Reprod Update* 2011b;**6**:772–790.

Ciavattini A, Carpini GD, Serri M, Vignini A, Sabbatinelli J, Tozzi A, Aggiusti A, Clemente N. Hypovitaminosis D and 'small burden' uterine fibroids: opportunity for a vitamin D supplementation. *Medicine (Baltimore)* 2016;**52**:e5698.

Colnot CL, Thompson Z, Miclau T, Werb Z, Helms JA. Altered fracture repair in the absence of MMP9. *Development* 2003;**17**:4123–4133.

Courtoy GE, Donnez J, Marbaix E, Dolmans M-M. In vivo mechanisms of uterine myoma volume reduction with ulipristal acetate treatment. *Fertil Steril* 2015;**2**:426–434.e1.

Cramer SF, Patel A. The frequency of uterine leiomyomas. *Am J Clin Pathol* 1990;**4**:435–438.

Day Baird D, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. *Am J Obstet Gynecol* 2003;**1**:100–107.

De Jonge MJA, Dumez H, Verweij J, Yarkoni S, Snyder D, Lacombe D, Marreaud S, Yamaguchi T, Punt CJA, Van Oosterom A. Phase I and pharmacokinetic study of halofuginone, an oral quinazolinone derivative in patients with advanced solid tumours. *Eur J Cancer* 2006;**12**:1768–1774.

Denis L, Debruyne F, De Porre P, Bruynseels J. Early clinical experience with liarozole (Liazal™) in patients with progressive prostate cancer. *Eur J Cancer* 1998;**4**:469–475.

Desmouliere A, Chaponnier C, Gabbiani G. Tissue repair, contraction, and the myofibroblast. *Wound Repair Regen* 2005;**1**:7–12.

Desmoulière A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-β1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol* 1993;**1**:103–111.

Desmouliere A, Redard M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *Am J Pathol* 1995;**1**:56–66.

Dimitrova IK, Richer JK, Rudolph MC, Spoelstra NS, Reno EM, Medina TM, Bradford AP. Gene expression profiling of multiple leiomyomata uteri and matched normal tissue from a single patient. *Fertil Steril* 2009;**6**:2650–2663.

Ding L, Luo X, Chegini N. The expression of IL-13 and IL-15 in leiomyoma and myometrium and their influence on TGF-β and proteases expression in leiomyoma and myometrial smooth muscle cells and SKLM, leiomyosarcoma cell line. *J Soc Gynecol Investig* 2004a;**1**:319A.

Ding L, Xu J, Luo X, Chegini N. Gonadotropin releasing hormone and transforming growth factor β activate mitogen-activated protein kinase/extracellularly regulated kinase and differentially regulate fibronectin, type I collagen, and plasminogen activator inhibitor-1 expression in leiomyoma and myometrial smooth muscle cells. *J Clin Endocrinol Metab* 2004b;**11**:5549–5557.

Dixon D, He H, Haseman JK. Immunohistochemical localization of growth factors and their receptors in uterine leiomyomas and matched myometrium. *Environ Health Perspect* 2000;**108**:795–802.

Doerr ME, Jones JI. The roles of integrins and extracellular matrix proteins in the insulin-like growth factor I-stimulated chemotaxis of human breast cancer cells. *J Biol Chem* 1996;**5**:2443–2447.

Donnez J, Hudecek R, Donnez O, Matule D, Arhendt HJ, Zatik J, Kasilovskiene Z, Dumitrascu MC, Fernandez H, Barlow DH *et al*. Efficacy and safety of repeated use of ulipristal acetate in uterine fibroids. *Fertil Steril* 2015;**2**:519–527. e3.

Donnez J, Tatarchuk TF, Bouchard P, Puscasiu L, Zakharenko NF, Ivanova T, Ugocsai G, Mara M, Jilla MP, Bestel E. Ulipristal acetate versus placebo for fibroid treatment before surgery. *N Engl J Med* 2012a;**5**:409–420.

Donnez J, Tomaszewski J, Vázquez F, Bouchard P, Lemieszczuk B, Baró F, Nouri K, Selvaggi L, Sodowski K, Bestel E. Ulipristal acetate versus .euprolide acetate for uterine fibroids. *N Engl J Med* 2012b;**5**:421–432.

Dou Q, Tarnuzzer RW, Williams RS, Schultz GS, Chegini N. Differential expression of matrix metalloproteinases and their tissue inhibitors in leiomyomata: a mechanism for gonadotrophin releasing hormone agonist-induced tumour regression. *Mol Hum Reprod* 1997;**11**:1005–1014.

Dou Q, Zhao Y, Tarnuzzer RW, Rong H, Williams RS, Schultz GS, Chegini N. Suppression of transforming growth factor-beta (TGF beta) and TGF beta receptor messenger ribonucleic acid and protein expression in leiomyomata in women receiving gonadotropin-releasing hormone agonist therapy. *J Clin Endocrinol Metab* 1996;**9**:3222–3230.

Droguett R, Cabello-Verrugio C, Riquelme C, Brandan E. Extracellular proteoglycans modify TGF-β bio-availability attenuating its signaling during skeletal muscle differentiation. *Matrix Biol* 2006;**6**:332–341.

Duscher D, Maan ZN, Wong VW, Rennert RC, Januszyk M, Rodrigues M, Hu M, Whitmore AJ, Whittam AJ, Longaker MT. Mechanotransduction and fibrosis. *J Biomech* 2014;**9**:1997–2005.

Eichenlaub-Ritter U, Winterscheidt U, Vogt E, Shen Y, Tinneberg H-R, Sorensen R. 2-Methoxyestradiol induces spindle aberrations, chromosome congression failure, and nondisjunction in mouse oocytes. *Biol Reprod* 2007; **5**:784–793.

Engel JB, Audebert A, Frydman R, Zivny J, Diedrich K. Presurgical short term treatment of uterine fibroids with different doses of cetrorelix acetate: a double-blind, placebo-controlled multicenter study. *Eur J Obstet Gynecol Reprod Biol* 2007;**2**:225–232.

Esteve JLC, Acosta R, Pérez Y, Rodriguez B, Seigler I, Sanchez C, Tomasi G. Mifepristone versus placebo to treat uterine myoma: a double-blind, randomized clinical trial. *Int J Womens Health* 2013;**5**:361–369.

Esteve JLC, Acosta R, Perez Y, Campos R, Hernandez AV, Texido CS. Treatment of uterine myoma with 5 or 10mg mifepristone daily during 6 months, post-treatment evolution over 12 months: double-blind randomised clinical trial. *Eur J Obstet Gynecol Reprod Biol* 2012;**2**:202–208.

Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR, Stakkestad J. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. *JAMA* 1999;**7**:637–645.

Eyden BP, Hale RJ, Richmond I, Buckley CH. Cytoskeletal filaments in the smooth muscle cells of uterine leiomyomata and myometrium: an ultrastructural and immunohistochemical analysis. *Virchows Arch A Pathol Anat Histopathol* 1992;**1**: 51–58.

Felberbaum RE, Küpker W, Krapp M, Gehl B, Ludwig M, Diedrich K. Preoperative reduction of uterine fibroids in only 16 days by administration of a gonadotrophin-releasing hormone antagonist (Cetrotide). *Reprod Biomed Online* 2001;**1**:14–18.

Feng L, Jayes FL, Johnson LN, Schomberg DW, Leppert PC. Biochemical pathways and myometrial cell differentiation leading to nodule formation containing collagen and fibronectin. *Curr Protein Pept Sci* 2016;**2**:155–166.

Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13α2 receptor is involved in induction of TGF-β1 production and fibrosis. *Nat Med* 2005;**1**:99–106.

Fiedler LR, Schönherr E, Waddington R, Niland S, Seidler DG, Aeschlimann D, Eble JA. Decorin regulates endothelial cell motility on collagen I through activation of insulin-like growth factor I receptor and modulation of α2β1 integrin activity. *J Biol Chem* 2008;**25**:17406–17415.

Fletcher NM, Saed MG, Abu-Soud HM, Al-Hendy A, Diamond MP, Saed GM. Uterine fibroids are characterized by an impaired antioxidant cellular system: potential role of hypoxia in the pathophysiology of uterine fibroids. *J Assist Reprod Genet* 2013;**7**:969–974.

Font B, Eichenberger D, Goldschmidt D, Boutillon MM, Hulmes DJS. Structural requirements for fibromodulin binding to collagen and the control of type I collagen fibrillogenesis. *Eur J Biochem* 1998;**3**:580–587.

Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. *J Pathol* 2003;**4**:500–503.

Gallagher CS, Morton CC. Genetic association studies in uterine fibroids: risk alleles presage the path to personalized therapies. *Semin Reprod Med* 2016;**34**: 235–241.

Gallagher WM, Currid CA, Whelan LC. Fibulins and cancer: friend or foe? *Trends Mol Med* 2005;**7**:336–340.

Geiger B, Bershadsky A, Pankov R, Yamada KM. Transmembrane crosstalk between the extracellular matrix and the cytoskeleton. *Nat Rev Mol Cell Biol* 2001;**11**:793–805.

Gentry CC, Okolo SO, Fong LF, Crow JC, Maclean AB, Perrett CW. Quantification of vascular endothelial growth factor-A in leiomyomas and adjacent myometrium. *Clin Sci (Lond)* 2001;**6**:691–695.

Giannandrea M, Parks WC. Diverse functions of matrix metalloproteinases during fibrosis. *Dis Model Mech* 2014;**2**:193–203.

Gilden M, Malik M, Britten J, Delgado T, Levy G, Catherino WH. Leiomyoma fibrosis inhibited by liarozole, a retinoic acid metabolic blocking agent. *Fertil Steril* 2012;**4**:1557–1562.

Giri SN, Hyde DM, Braun RK, Gaarde W, Harper JR, Pierschbacher MD. Antifibrotic effect of decorin in a bleomycin hamster model of lung fibrosis. *Biochem Pharmacol* 1997;**11**:1205–1216.

Gonzalez-Barcena D, Alvarez RB, Ochoa EP, Cornejo IC, Comaru-Schally AM, Schally AV, Engel J, Reissmann T, Riethmuller-Winzen H. Treatment of uterine leiomyomas with luteinizing hormone-releasing hormone antagonist Cetrorelix. *Hum Reprod* 1997;**9**:2028–2035.

Goss PE, Strasser K, Marques R, Clemons M, Oza A, Goel R, Blackstein M, Kaizer L, Sterns EE, Nabholtz J-M. Liarozole fumarate (R85246): in the treatment of ER negative, tamoxifen refractory or chemotherapy resistant postmenopausal metastatic breast cancer. *Breast Cancer Res Treat* 2000;**2**:177–188.

Grudzien MM, Low PS, Manning PC, Arredondo M, Belton RJ, Nowak RA. The antifibrotic drug halofuginone inhibits proliferation and collagen production by human leiomyoma and myometrial smooth muscle cells. *Fertil Steril* 2010;**4**: 1290–1298.

Guo J, Gu N, Chen J, Shi T, Zhou Y, Rong Y, Zhou T, Yang W, Cui X, Chen W. Neutralization of interleukin-1 beta attenuates silica-induced lung inflammation and fibrosis in C57BL/6 mice. *Arch Toxicol* 2013;**11**:1963–1973.

Guo XC, Segars JH. The impact and management of fibroids for fertility: an evidence-based approach. *Obstet Gynecol Clin North Am* 2012;**4**:521–533.

Gupta SC, Patchva S, Aggarwal BB. Therapeutic roles of curcumin: lessons learned from clinical trials. *AAPS J* 2013;**1**:195–218.

Halder SK, Goodwin JS, Al-Hendy A. 1,25-Dihydroxyvitamin D reduces TGF-β3-induced fibrosis-related gene expression in human uterine leiomyoma cells. *J Clin Endocrinol Metab* 2011;**4**:E754–E762.

Halder SK, Osteen KG, Al-Hendy A. 1, 25-dihydroxyvitamin d3 reduces extracellular matrix-associated protein expression in human uterine fibroid cells. *Biol Reprod* 2013;**6**:150.

Halder SK, Sharan C, Al-Hendy A. 1,25-dihydroxyvitamin D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model. *Biol Reprod* 2012;**4**:116.

Hanks SK, Calalb MB, Harper MC, Patel SK. Focal adhesion protein-tyrosine kinase phosphorylated in response to cell attachment to fibronectin. *Proc Natl Acad Sci USA* 1992;**18**:8487–8491.

Harrison-Woolrych ML, Sharkey AM, Charnock-Jones DS, Smith SK. Localization and quantification of vascular endothelial growth factor messenger ribonucleic acid in human myometrium and leiomyomata. *J Clin Endocrinol Metab* 1995;**6**: 1853–1858.

Harrison MR, Hahn NM, Pili R, Oh WK, Hammers H, Sweeney C, Kim K, Perlman S, Arnott J, Sidor C. A phase II study of 2-methoxyestradiol (2ME2) NanoCrystal® dispersion (NCD) in patients with taxane-refractory, metastatic castrate-resistant prostate cancer (CRPC). *Invest New Drugs* 2011;**6**:1465–1474.

Hassan MH, Eyzaguirre E, Arafa HMM, Hamada FMA, Salama SA, Al-Hendy A. Memy I: a novel murine model for uterine leiomyoma using adenovirus-enhanced human fibroid explants in severe combined immune deficiency mice. *Am J Obstet Gynecol* 2008;**2**:156. e1–156. e8.

Hassan MH, Salama SA, Arafa HM, Hamada FM, Al-Hendy A. Adenovirus-mediated delivery of a dominant-negative estrogen receptor gene in uterine leiomyoma cells abrogates estrogen- and progesterone-regulated gene expression. *J Clin Endocrinol Metab* 2007;**10**:3949–3957.

Hedbom E, Heinegård D. Binding of fibromodulin and decorin to separate sites on fibrillar collagens. *J Biol Chem* 1993;**36**:27307–27312.

Herndon CN, Aghajanova L, Bayalan S, Erikson D, Barragan F, Goldfien G, Vo KC, Hawkins S, Giudice LC. Global transcriptome abnormalities of the eutopic endometrium from women with adenomyosis. *Reprod Sci* 2016;**10**:1289–1303.

Higgins DF, Kimura K, Bernhardt WM, Shrimanker N, Akai Y, Hohenstein B, Saito Y, Johnson RS, Kretzler M, Cohen CD. Hypoxia promotes fibrogenesis in vivo via HIF-1 stimulation of epithelial-to-mesenchymal transition. *J Clin Invest* 2007; **12**:3810–3820.

Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. *Am J Pathol* 2007;**6**:1807–1816.

Ho YY, Lagares D, Tager AM, Kapoor M. Fibrosis-a lethal component of systemic sclerosis. *Nat Rev Rheumatol* 2014;**7**:390–402.

Hoekstra AV, Sefton EC, Berry E, Lu Z, Hardt J, Marsh E, Yin P, Clardy J, Chakravarti D, Bulun S. Progestins activate the AKT pathway in leiomyoma cells and promote survival. *J Clin Endocrinol Metab* 2009;**5**:1768–1774.

Holdsworth-Carson SJ, Zaitseva M, Vollenhoven BJ, Rogers PAW. Clonality of smooth muscle and fibroblast cell populations isolated from human fibroid and myometrial tissues. *Mol Hum Reprod* 2014;**3**:250–259.

Huang H, Kamm RD, Lee RT. Cell mechanics and mechanotransduction: pathways, probes, and physiology. *Am J Physiol Cell Physiol* 2004;**1**:C1–C11.

Hwu YM, Li SH, Lee RK, Tsai YH, Yeh TS, Lin SY. Increased expression of platelet-derived growth factor C messenger ribonucleic acid in uterine leiomyomata. *Fertil Steril* 2008;**2**:468–471.

Iimuro Y, Nishio T, Morimoto T, Nitta T, Stefanovic B, Choi SK, Brenner DA, Yamaoka Y. Delivery of matrix metalloproteinase-1 attenuates established liver fibrosis in the rat. *Gastroenterology* 2003;**2**:445–458.

Ikenaka Y, Yoshiji H, Kuriyama S, Yoshii J, Noguchi R, Tsujinoue H, Yanase K, Namisaki T, Imazu H, Masaki T. Tissue inhibitor of metalloproteinases-1 (TIMP-1) inhibits tumor growth and angiogenesis in the TIMP-1 transgenic mouse model. *Int J Cancer* 2003;**3**:340–346.

Islam MS, Catherino WH, Protic O, Janjusevic M, Gray PC, Giannubilo SR, Ciavattini A, Lamanna P, Tranquilli AL, Petraglia F *et al*. Role of activin-A and myostatin and their signaling pathway in human myometrial and leiomyoma cell function. *J Clin Endocrinol Metab* 2014a;**5**:E775–E785.

Islam MS, Greco S, Janjusevic M, Ciavattini A, Giannubilo SR, D'Adderio A, Biagini A, Fiorini R, Castellucci M, Ciarmela P. Growth factors and pathogenesis. *Best Pract Res Clin Obstet Gynaecol* 2016;**34**:25–36.

Islam MS, Protic O, Ciavattini A, Giannubilo SR, Tranquilli AL, Catherino WH, Castellucci M, Ciarmela P. Tranilast, an orally active antiallergic compound, inhibits extracellular matrix production in human uterine leiomyoma and myometrial cells. *Fertil Steril* 2014b;**2**:597–606.

Islam MS, Protic O, Giannubilo S, Stortoni P, Ciavattini A, Lamanna P, Tranquilli AL, Castellucci M, Ciarmela P. Antiproliferative effect of tranilast on human myometrial and leiomyoma cells. *Biol Biomed Rep* 2012;**5**:321–327.

Islam MS, Protic O, Stortoni P, Grechi G, Lamanna P, Petraglia F, Castellucci M, Ciarmela P. Complex networks of multiple factors in the pathogenesis of uterine leiomyoma. *Fertil Steril* 2013a;**1**:178–193.

Islam MS, Protic O, Toti P, Giannubilo SR, Tranquilli AL, Petraglia F, Castellucci M, Ciarmela P. Uterine leiomyoma: available medical treatments and new possible therapeutic options. *J Clin Endocrinol Metab* 2013b;**3**:921–934.

Itoh Y. MT1-MMP: A key regulator of cell migration in tissue. *IUBMB life* 2006;**10**: 589–596.

Iwahashi M, Muragaki Y. Increased type I and V collagen expression in uterine leiomyomas during the menstrual cycle. *Fertil Steril* 2011;**6**:2137–2139.

Iwahashi M, Muragaki Y, Ikoma M, Mabuchi Y, Kobayashi AYA, Tanizaki Y, Ino K. Immunohistochemical analysis of collagen expression in uterine leiomyomata during the menstrual cycle. *Exp Ther Med* 2010;**2**:287–290.

Jakubowska K, Pryczynicz A, Iwanowicz P, Niewiński A, Maciorkowska E, Hapanowicz J, Jagodzińska D, Kemona A, Guzińska-Ustymowicz K. Expressions of matrix metalloproteinases (MMP-2, MMP-7, and MMP-9) and their inhibitors (TIMP-1, TIMP-2) in inflammatory bowel diseases. *Gastroenterol Res Pract* 2016; **2016**:2456179.

Jayes FL, Liu B, Moutos FT, Kuchibhatla M, Guilak F, Leppert PC. Loss of stiffness in collagen-rich uterine fibroids after digestion with purified collagenase Clostridium histolyticum. *Am J Obstet Gynecol* 2016;**5**:596.e1–596.e8.

Jirecek S, Lee A, Pavo I, Crans G, Eppel W, Wenzl R. Raloxifene prevents the growth of uterine leiomyomas in premenopausal women. *Fertil Steril* 2004;**1**: 132–136.

Jirouskova M, Zbodakova O, Gregor M, Chalupsky K, Sarnova L, Hajduch M, Ehrmann J, Jirkovska M, Sedlacek R. Hepatoprotective effect of MMP-19 deficiency in a mouse model of chronic liver fibrosis. *PloS one* 2012;**10**:e46271.

Jones JL, Walker RA. Integrins: a role as cell signalling molecules. *Mol Pathol* 1999; **4**:208.

Joseph DS, Malik M, Nurudeen S, Catherino WH. Myometrial cells undergo fibrotic transformation under the influence of transforming growth factor β-3. *Fertil Steril* 2010;**5**:1500–1508.

Kashani BN, Centini G, Morelli SS, Weiss G, Petraglia F. Role of medical management for uterine leiomyomas. *Best Pract Res Clin Obstet Gynaecol* 2016;**34**:85–103.

Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J* 1990;**5**:1603–1614.

Kawaguchi K, Fujii S, Konishi I, Nanbu Y, Nonogaki H, Mori T. Mitotic activity in uterine leiomyomas during the menstrual cycle. *Am J Obstet Gynecol* 1989;**3**:637–641.

Ke X, Dou F, Cheng Z, Dai H, Zhang W, Qu X, Ding P, Zuo X. High expression of cyclooxygenase-2 in uterine fibroids and its correlation with cell proliferation. *Eur J Obstet Gynecol Reprod Biol* 2013;**2**:199–203.

Kino T, Takatori H, Manoli I, Wang Y, Tiulpakov A, Blackman MR, Su YA, Chrousos GP, DeCherney AH, Segars JH. Brx mediates the response of lymphocytes to osmotic stress through the activation of NFAT5. *Sci Signal* 2009;**57**:ra5.

Kjerulff KH, Langenberg P, Seidman JD, Stolley PD, Guzinski GM. Uterine leiomyomas. Racial differences in severity, symptoms and age at diagnosis. *J Reprod Med* 1996;**7**:483–490.

Klein T, Bischoff R. Physiology and pathophysiology of matrix metalloproteases. *Amino Acids* 2011;**2**:271–290.

Kleinman HK, Cannon FB, Laurie GW, Hassell JR, Aumailley M, Terranova VP, Martin GR, DuBois-Dalcq M. Biological activities of laminin. *J Cell Biochem* 1985; **4**:317–325.

Konneh M. Tranilast Kissei Pharmaceutical. *IDrugs* 1998;**1**:141–146.

Koohestani F, Braundmeier AG, Mahdian A, Seo J, Bi J, Nowak RA. Extracellular matrix collagen alters cell proliferation and cell cycle progression of human uterine leiomyoma smooth muscle cells. *PloS one* 2013;**9**:e75844.

Koohestani F, Qiang W, MacNeill AL, Druschitz SA, Serna VA, Adur M, Kurita T, Nowak RA. Halofuginone suppresses growth of human uterine leiomyoma cells in a mouse xenograft model. *Hum Reprod* 2016;**7**:1540–1551.

Korompelis P, Piperi C, Adamopoulos C, Dalagiorgou G, Korkolopoulou P, Sepsa A, Antsaklis A, Papavassiliou AG. Expression of vascular endothelial factor-A, gelatinases (MMP-2, MMP-9) and TIMP-1 in uterine leiomyomas. *Clin Chem Lab Med* 2015;**9**:1415–1424.

Kovacs KA, Oszter A, Gocze PM, Kornyei JL, Szabo I. Comparative analysis of cyclin D1 and oestrogen receptor (α and β) levels in human leiomyoma and adjacent myometrium. *Mol Hum Reprod* 2001;**11**:1085–1091.

Kurachi O, Matsuo H, Samoto T, Maruo T. Tumor necrosis factor-α expression in human uterine leiomyoma and its down-regulation by progesterone. *J Clin Endocrinol Metab* 2001;**5**:2275–2280.

Kurzrock R, Estey E, Talpaz M. All-trans retinoic acid: tolerance and biologic effects in myelodysplastic syndrome. *J Clin Oncol* 1993;**8**:1489–1495.

Lee BS, Margolin SB, Nowak RA. Pirfenidone: a novel pharmacological agent that inhibits leiomyoma cell proliferation and collagen production. *J Clin Endocrinol Metab* 1998;**1**:219–223.

Lee CG, Homer RJ, Zhu Z, Lanone S, Wang X, Koteliansky V, Shipley JM, Gotwals P, Noble P, Chen Q. Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor β1. *J Exp Med* 2001;**6**:809–822.

Lee S, Jilani SM, Nikolova GV, Carpizo D, Iruela-Arispe ML. Processing of VEGF-A by matrix metalloproteinases regulates bioavailability and vascular patterning in tumors. *J Cell Biol* 2005;**4**:681–691.

Leibovich SJ, Ross R. The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. *Am J Pathol* 1975;**1**:71–100.

Leppert P, Fouany M, Segars JH. Understanding uterine fibroids. In: Segars JH (ed). *Fibroids*. Oxford: John Wiley & Sons, Ltd, 2013;1–10.

Leppert PC, Baginski T, Prupas C, Catherino WH, Pletcher S, Segars JH. Comparative ultrastructure of collagen fibrils in uterine leiomyomas and normal myometrium. *Fertil Steril* 2004;1182–1187.

Leppert PC, Catherino WH, Segars JH. A new hypothesis about the origin of uterine fibroids based on gene expression profiling with microarrays. *Am J Obstet Gynecol* 2006;**2**:415–420.

Levens E, Luo X, Ding L, Williams RS, Chegini N. Fibromodulin is expressed in leiomyoma and myometrium and regulated by gonadotropin-releasing hormone analogue therapy and TGF-β through Smad and MAPK-mediated signalling. *Mol Hum Reprod* 2005;**7**:489–494.

Levy G, Malik M, Britten J, Gilden M, Segars J, Catherino WH. Liarozole inhibits transforming growth factor-β3-mediated extracellular matrix formation in human three-dimensional leiomyoma cultures. *Fertil Steril* 2014;**1**:272–281. e2.

Li G, Fridman R, Kim H-RC. Tissue inhibitor of metalloproteinase-1 inhibits apoptosis of human breast epithelial cells. *Cancer Res* 1999;**24**:6267–6275.

Liang M, Wang H, Zhang Y, Lu S, Wang Z. Expression and functional analysis of platelet-derived growth factor in uterine leiomyomata. *Cancer Biol Ther* 2006;**1**: 28–33.

Lindahl GE, Chambers RC, Papakrivopoulou J, Dawson SJ, Jacobsen MC, Bishop JE, Laurent GJ. Activation of fibroblast procollagen α1(I) transcription by mechanical strain is transforming growth factor-β-dependent and involves increased binding

of CCAAT-binding factor (CBF/NF-Y) at the proximal promoter. *J Biol Chem* 2002;**8**:6153–6161.

Löffek S, Schilling O, Franzke C-W. Biological role of matrix metalloproteinases: a critical balance. *Eur Respir J* 2011;**38**:191–208.

Logan A, Baird A, Berry M. Decorin attenuates gliotic scar formation in the rat cerebral hemisphere. *Exp Neurol* 1999;**2**:504–510.

Luo N, Guan Q, Zheng L, Qu X, Dai H, Cheng Z. Estrogen-mediated activation of fibroblasts and its effects on the fibroid cell proliferation. *Transl Res* 2014;**3**:232–241.

Luo X, Chegini N. The expression and potential regulatory function of microRNAs in the pathogenesis of leiomyoma. *Semin Reprod Med* 2008;**6**:500–514.

Luo X, Ding L, Chegini N. CCNs, fibulin-1C and S100A4 expression in leiomyoma and myometrium: inverse association with TGF-β and regulation by TGF-β in leiomyoma and myometrial smooth muscle cells. *Mol Hum Reprod* 2006;**4**:245–256.

Luo X, Ding L, Xu J, Chegini N. Gene expression profiling of leiomyoma and myometrial smooth muscle cells in response to transforming growth factor-β. *Endocrinology* 2005;**3**:1097–1118.

Luo X, Yin P, Reierstad S, Ishikawa H, Lin Z, Pavone ME, Zhao H, Marsh EE, Bulun SE. Progesterone and mifepristone regulate L-type amino acid transporter 2 and 4F2 heavy chain expression in uterine leiomyoma cells. *J Clin Endocrinol Metab* 2009;**11**:4533–4539.

Mahabeleshwar GH, Feng W, Reddy K, Plow EF, Byzova TV. Mechanisms of integrin-vascular endothelial growth factor receptor cross-activation in angiogenesis. *Circ Res* 2007;**6**:570–580.

Malik M, Britten J, Cox J, Patel A, Catherino WH. Gonadotropin-releasing hormone analogues inhibit leiomyoma extracellular matrix despite presence of gonadal hormones. *Fertil Steril* 2016;**1**:214–224.

Malik M, Britten J, Segars J, Catherino WH. Leiomyoma cells in 3-dimensional cultures demonstrate an attenuated response to fasudil, a rho-kinase inhibitor, when compared to 2-dimensional cultures. *Reprod Sci* 2014;**9**:1126–1138.

Malik M, Catherino WH. Novel method to characterize primary cultures of leiomyoma and myometrium with the use of confirmatory biomarker gene arrays. *Fertil Steril* 2007;**5**:1166–1172.

Malik M, Mendoza M, Payson M, Catherino WH. Curcumin, a nutritional supplement with antineoplastic activity, enhances leiomyoma cell apoptosis and decreases fibronectin expression. *Fertil Steril* 2009;**5** Suppl:2177–2184.

Malik M, Norian J, McCarthy-Keith D, Britten J, Catherino WH. Why leiomyomas are called fibroids: the central role of extracellular matrix in symptomatic women. *Semin Reprod Med* 2010;**3**:169–179.

Malik M, Segars J, Catherino WH. Integrin β1 regulates leiomyoma cytoskeletal integrity and growth. *Matrix Biol* 2012;**7-8**:389–397.

Malik M, Webb J, Catherino WH. Retinoic acid treatment of human leiomyoma cells transformed the cell phenotype to one strongly resembling myometrial cells. *Clin Endocrinol (Oxf)* 2008;**3**:462–470.

Mangrulkar RS, Ono M, Ishikawa M, Takashima S, Klagsbrun M, Nowak RA. Isolation and characterization of heparin-binding growth factors in human leiomyomas and normal myometrium. *Biol Reprod* 1995;**3**:636–646.

Manicone AM, Huizar I, McGuire JK. Matrilysin (matrix metalloproteinase-7) regulates anti-inflammatory and antifibrotic pulmonary dendritic cells that express CD103 (α E β 7-integrin). *Am J Pathol* 2009;**6**:2319–2331.

Maniotis AJ, Chen CS, Ingber DE. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc Natl Acad Sci USA* 1997;**3**:849–854.

Mannello F, Luchetti F, Falcieri E, Papa S. Multiple roles of matrix metalloproteinases during apoptosis. *Apoptosis* 2005;**1**:19–24.

Marsh EE, Chibber S, Wu J, Siegersma K, Kim J, Bulun S. Epidermal growth factor-containing fibulin-like extracellular matrix protein 1 expression and regulation in uterine leiomyoma. *Fertil Steril* 2016a;**4**:1070–1075.

Marsh EE, Steinberg ML, Parker JB, Wu J, Chakravarti D, Bulun SE. Decreased expression of microRNA-29 family in leiomyoma contributes to increased major fibrillar collagen production. *Fertil Steril* 2016b;**3**:766–772.

Marshall LM, Spiegelman D, Barbieri RL, Goldman MB, Manson JE, Colditz GA, Willett WC, Hunter DJ. Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. *Obstet Gynecol* 1997;**6**:967–973.

Martino S, Disch D, Dowsett SA, Keech CA, Mershon JL. Safety assessment of raloxifene over eight years in a clinical trial setting. *Curr Med Res Opin* 2005;**9**:1441–1452.

Maruo T, Ohara N, Wang J, Matsuo H. Sex steroidal regulation of uterine leiomyoma growth and apoptosis. *Hum Reprod Update* 2004;**3**:207–220.

Mas A, Nair S, Laknaur A, Simon C, Diamond MP, Al-Hendy A. Stro-1/CD44 as putative human myometrial and fibroid stem cell markers. *Fertil Steril* 2015;**1**:225–234.e3.

Matrisian LM. The matrix-degrading metalloproteinases. *Bioessays* 1992;**7**:455–463.

Matsumoto S-i, Katoh M, Saito S, Watanabe T, Masuho Y. Identification of soluble type of membrane-type matrix metalloproteinase-3 formed by alternatively spliced mRNA. *Biochim Biophys Acta* 1997;**2**:159–170.

McCarthy-Keith DM, Malik M, Britten J, Segars J, Catherino WH. Gonadotropin-releasing hormone agonist increases expression of osmotic response genes in leiomyoma cells. *Fertil Steril* 2011;**7**:2383–2387.

McCawley LJ, Matrisian LM. Matrix metalloproteinases: they're not just for matrix anymore! *Curr Opin Cell Biol* 2001;**5**:534–540.

Melis GB, Piras B, Marotto MF, Orru MM, Maricosu G, Pilloni M, Guerriero S, Angiolucci M, Lello S, Paoletti AM. Pharmacokinetic evaluation of ulipristal acetate for uterine leiomyoma treatment. *Expert Opin Drug Metab Toxicol* 2012;**7**:901–908.

Mesquita FS, Dyer SN, Heinrich DA, Bulun SE, Marsh EE, Nowak RA. Reactive oxygen species mediate mitogenic growth factor signaling pathways in human leiomyoma smooth muscle cells. *Biol Reprod* 2010;**2**:341–351.

Mitropoulou TN, Theocharis AD, Stagiannis KD, Karamanos NK. Identification, quantification and fine structural characterization of glycosaminoglycans from uterine leiomyoma and normal myometrium. *Biochimie* 2001;**6**:529–536.

Mitsiades N, Yu W-H, Poulaki V, Tsokos M, Stamenkovic I. Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity. *Cancer Res* 2001;**2**:577–581.

Moore AB, Yu L, Swartz CD, Zheng X, Wang L, Castro L, Kissling GE, Walmer DK, Robboy SJ, Dixon D. Human uterine leiomyoma-derived fibroblasts stimulate uterine leiomyoma cell proliferation and collagen type I production, and activate RTKs and TGF beta receptor signaling in coculture. *Cell Commun Signal* 2010;**8**:10.

Morikawa A, Ohara N, Xu Q, Nakabayashi K, DeManno DA, Chwalisz K, Yoshida S, Maruo T. Selective progesterone receptor modulator asoprisnil down-regulates collagen synthesis in cultured human uterine leiomyoma cells through up-regulating extracellular matrix metalloproteinase inducer. *Hum Reprod* 2008;**4**:944–951.

Mui KL, Chen CS, Assoian RK. The mechanical regulation of integrin–cadherin crosstalk organizes cells, signaling and forces. *J Cell Sci* 2016;**6**:1093–1100.

Murphy FR, Issa R, Zhou X, Ratnarajah S, Nagase H, Arthur MJ, Benyon C, Iredale JP. Inhibition of apoptosis of activated hepatic stellate cells by tissue inhibitor of metalloproteinase-1 is mediated via effects on matrix metalloproteinase inhibition implications for reversibility of liver fibrosis. *J Biol Chem* 2002;**13**:11069–11076.

Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 2006;**3**:562–573.

Nair S, Al-Hendy A. Adipocytes enhance the proliferation of human leiomyoma cells via TNF-α proinflammatory cytokine. *Reprod Sci* 2011;**12**:1186–1192.

Nakamura T, Sugino K, Titani K, Sugino H. Follistatin, an activin-binding protein, associates with heparan sulfate chains of proteoglycans on follicular granulosa cells. *J Biol Chem* 1991;**29**:19432–19437.

Nelissen I, Martens E, Van den Steen PE, Proost P, Ronsse I, Opdenakker G. Gelatinase B/matrix metalloproteinase-9 cleaves interferonon-β and is a target for immunotherapy. *Brain* 2003;**6**:1371–1381.

Nierth-Simpson EN, Martin MM, Chiang TC, Melnik LI, Rhodes LV, Muir SE, Burow ME, McLachlan JA. Human uterine smooth muscle and leiomyoma cells differ in their rapid 17β-estradiol signaling: implications for proliferation. *Endocrinology* 2009;**5**:2436–2445.

Nilsson S, Makela S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JÃ. Mechanisms of estrogen action. *Physiol Rev* 2001;**4**:1535–1565.

Nishizuka I, Ichikawa Y, Ishikawa T, Kamiyama M, Hasegawa S, Momiyama N, Miyazaki K, Shimada H. Matrilysin stimulates DNA synthesis of cultured vascular endothelial cells and induces angiogenesis in vivo. *Cancer Lett* 2001;**2**:175–182.

Norian JM, Malik M, Parker CY, Joseph D, Leppert PC, Segars JH, Catherino WH. Transforming growth factor β3 regulates the versican variants in the extracellular matrix-rich uterine leiomyomas. *Reprod Sci* 2009;**12**:1153–1164.

Norian JM, Owen CM, Taboas J, Korecki C, Tuan R, Malik M, Catherino WH, Segars JH. Characterization of tissue biomechanics and mechanical signaling in uterine leiomyoma. *Matrix Biol* 2012;**1**:57–65.

Ockleford C, Bright N, Hubbard A, D'Lacey C, Smith J, Gardiner L, Sheikh T, Albentosa M, Turtle K. Micro-trabeculae, macro-plaques or mini-basement membranes in human term fetal membranes? *Philos Trans R Soc Lond B Biol Sci* 1993;**1300**:121–136.

Oh J, Seo D-W, Diaz T, Wei B, Ward Y, Ray JM, Morioka Y, Shi S, Kitayama H, Takahashi C. Tissue inhibitors of metalloproteinase 2 inhibits endothelial cell migration through increased expression of RECK. *Cancer Res* 2004;**24**:9062–9069.

Okolo S. Incidence, aetiology and epidemiology of uterine fibroids. *Best Pract Res Clin Obstet Gynaecol* 2008;**4**:571–588.

Ono M, Maruyama T, Masuda H, Kajitani T, Nagashima T, Arase T, Ito M, Ohta K, Uchida H, Asada H. Side population in human uterine myometrium displays phenotypic and functional characteristics of myometrial stem cells. *Proc Natl Acad Sci USA* 2007;**47**:18700–18705.

Onozuka I, Kakinuma S, Kamiya A, Miyoshi M, Sakamoto N, Kiyohashi K, Watanabe T, Funaoka Y, Ueyama M, Nakagawa M. Cholestatic liver fibrosis and toxin-induced fibrosis are exacerbated in matrix metalloproteinase-2 deficient mice. *Biochem Biophys Res Commun* 2011;**1**:134–140.

Ortéga N, L'Faqihi F-E, Plouët J. Control of vascular endothelial growth factor angiogenic activity by the extracellular matrix. *Biol Cell* 1998;**5**:381–390.

Paffoni A, Somigliana E, Vigano P, Benaglia L, Cardellicchio L, Pagliardini L, Papaleo E, Candiani M, Fedele L. Vitamin D status in women with uterine leiomyomas. *J Clin Endocrinol Metab* 2013;**8**:E1374–E1378.

Palmer SS, Haynes-Johnson D, Diehl T, Nowak RA. Increased expression of stromelysin 3 mRNA in leiomyomas (uterine fibroids) compared with myometrium. *J Soc Gynecol Investig* 1998;**4**:203–209.

Pankov R, Yamada KM. Fibronectin at a glance. *J Cell Sci* 2002;**20**:3861–3863.

Park JE, Keller G-A, Ferrara N. The vascular endothelial growth factor (VEGF) isoforms: differential deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. *Mol Biol Cell* 1993;**12**:1317–1326.

Park SB, Jee BC, Kim SH, Cho YJ, Han M. Cyclooxygenase-2 inhibitor, celecoxib, inhibits leiomyoma cell proliferation through the nuclear factor κB pathway. *Reprod Sci* 2014;**9**:1187–1195.

Parsons JT. Focal adhesion kinase: the first ten years. *J Cell Sci* 2003;**8**:1409–1416.

PaszeK MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D. Tensional homeostasis and the malignant phenotype. *Cancer cell* 2005;**3**:241–254.

Patel A, Malik M, Britten J, Cox J, Catherino WH. Mifepristone inhibits extracellular matrix formation in uterine leiomyoma. *Fertil Steril* 2016;**4**:1102–1110.

Pei D. Identification and characterization of the fifth membrane-type matrix metalloproteinase MT5-MMP. *J Biol Chem* 1999;**13**:8925–8932.

Pickering JG. Regulation of vascular cell behavior by collagen form is function. *Circ Res* 2001;**5**:458–459.

Plewka A, Madej P, Plewka D, Kowalczyk A, Miskiewicz A, Wittek P, Leks T, Bilski R. Immunohistochemical localization of selected pro-inflammatory factors in uterine myomas and myometrium in women of various ages. *Folia Histochem Cytobiol* 2013;**1**:73–83.

Polacheck WJ, German AE, Mammoto A, Ingber DE, Kamm RD. Mechanotransduction of fluid stresses governs 3D cell migration. *Proc Natl Acad Sci USA* 2014;**7**:2447–2452.

Poli G. Pathogenesis of liver fibrosis: role of oxidative stress. *Mol Aspects Med* 2000;**3**:49–98.

Porter KE, Turner NA, O'Regan DJ, Balmforth AJ, Ball SG. Simvastatin reduces human atrial myofibroblast proliferation independently of cholesterol lowering via inhibition of RhoA. *Cardiovasc Res* 2004;**4**:745–755.

Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. I. Paracrine cells important in health and disease. *Am J Physiol Cell Physiol* 1999a;**1**:C1–C19.

Powell WC, Fingleton B, Wilson CL, Boothby M, Matrisian LM. The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. *Curr Biol* 1999b;**24**:1441–1447.

Protic O, Toti P, Islam MS, Occhini R, Giannubilo SR, Catherino WH, Janjusevic M, Lamanna P, Cinti S, Petraglia F *et al*. Possible involvement of inflammatory/reparative processes in the development of uterine fibroids. *Cell Tissue Res* 2016;**2**:415–427.

Qiang W, Liu Z, Serna VA, Druschitz SA, Liu Y, Espona-Fiedler M, Wei J-J, Kurita T. Down-regulation of miR-29b is essential for pathogenesis of uterine leiomyoma. *Endocrinology* 2014;**3**:663–669.

Raines EW, Ross R. Compartmentalization of PDGF on extracellular binding sites dependent on exon-6-encoded sequences. *J Cell Biol* 1992;**2**:533–543.

Rajkumar SV, Richardson PG, Lacy MQ, Dispenzieri A, Greipp PR, Witzig TE, Schlossman R, Sidor CF, Anderson KC, Gertz MA. Novel therapy with 2-methoxyestradiol for the treatment of relapsed and plateau phase multiple myeloma. *Clin Cancer Res* 2007;**20**:6162–6167.

Rapraeger AC, Krufka A, Olwin BB. Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science* 1991;**5013**:1705–1708.

Richards PA, Richards PD, Tiltman AJ. The ultrastructure of fibromyomatous myometrium and its relationship to infertility. *Hum Reprod Update* 1998;**5**:520–525.

Rodríguez D, Morrison CJ, Overall CM. Matrix metalloproteinases: what do they not do? New substrates and biological roles identified by murine models and proteomics. *Biochim Biophys Acta* 2010;**1**:39–54.

Rogers R, Norian J, Malik M, Christman G, Abu-Asab M, Chen F, Korecki C, Iatridis J, Catherino WH, Tuan RS. Mechanical homeostasis is altered in uterine leiomyoma. *Am J Obstet Gynecol* 2008;**4**:474. e1–474. e11.

Rohani MG, Parks WC. Matrix remodeling by MMPs during wound repair. *Matrix Biol* 2015;**44-46**:113–121.

Ruwhof C, van der Laarse A. Mechanical stress-induced cardiac hypertrophy: mechanisms and signal transduction pathways. *Cardiovasc Res* 2000;**1**:23–37.

Saksela O, Moscatelli D, Sommer A, Rifkin DB. Endothelial cell-derived heparan sulfate binds basic fibroblast growth factor and protects it from proteolytic degradation. *J Cell Biol* 1988;**2**:743–751.

Salama SA, Diaz-Arrastia CR, Kilic GS, Kamel MW. 2-Methoxyestradiol causes functional repression of transforming growth factor β3 signaling by ameliorating Smad and non-Smad signaling pathways in immortalized uterine fibroid cells. *Fertil Steril* 2012;**1**:178–184. e1.

Salama SA, Nasr AB, Dubey RK, Al-Hendy A. Estrogen metabolite 2-methoxyestradiol induces apoptosis and inhibits cell proliferation and collagen production in rat and human leiomyoma cells: a potential medicinal treatment for uterine fibroids. *J Soc Gynecol Investig* 2006;**8**:542–550.

Sanci M, Dikis C, Inan S, Turkoz E, Dicle N, Ispahi C. Immunolocalization of VEGF, VEGF receptors, EGF-R and Ki-67 in leiomyoma, cellular leiomyoma and leiomyosarcoma. *Acta Histochem* 2011;**3**:317–325.

Santulli P, Borghese B, Lemaréchal H, Leconte M, Millischer A-E, Batteux F, Chapron C, Borderie D. Increased serum oxidative stress markers in women with uterine leiomyoma. *PloS one* 2013;**8**:e72069.

Scaffidi AK, Petrovic N, Moodley YP, Fogel-Petrovic M, Kroeger KM, Seeber RM, Eidne KA, Thompson PJ, Knight DA. αvβ3 integrin interacts with the transforming growth factor β (TGFβ) type II receptor to potentiate the proliferative effects of TGFβ1 in living human lung fibroblasts. *J Biol Chem* 2004;**36**:37726–37733.

Schenk S, Hintermann E, Bilban M, Koshikawa N, Hojilla C, Khokha R, Quaranta V. Binding to EGF receptor of a laminin-5 EGF-like fragment liberated during MMP-dependent mammary gland involution. *J Cell Biol* 2003;**1**:197–209.

Schlaepfer DD, Mitra SK. Multiple connections link FAK to cell motility and invasion. *Curr Opin Genet Dev* 2004;**1**:92–101.

Schmidt G, Hausser H, Kresse H. Interaction of the small proteoglycan decorin with fibronectin. Involvement of the sequence NKISK of the core protein. *Biochem J* 1991;**280**:411–414.

Schneller M, Vuori K, Ruoslahti E. αvβ3 integrin associates with activated insulin and PDGFβ receptors and potentiates the biological activity of PDGF. *EMBO J* 1997;**18**:5600–5607.

Seo D-W, Li H, Guedez L, Wingfield PT, Diaz T, Salloum R, Wei B-Y, Stetler-Stevenson WG. TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. *Cell* 2003;**2**:171–180.

Seong J, Wang N, Wang Y. Mechanotransduction at focal adhesions: from physiology to cancer development. *J Cell Mol Med* 2013;**5**:597–604.

Serini G, Bochaton-Piallat M-L, Ropraz P, Geinoz A, Borsi L, Zardi L, Gabbiani G. The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-β1. *J Cell Biol* 1998;**3**:873–881.

Shen Q, Hua Y, Jiang W, Zhang W, Chen M, Zhu X. Effects of mifepristone on uterine leiomyoma in premenopausal women: a meta-analysis. *Fertil Steril* 2013; **6**:1722–1726. e10.

Shi Y, Massagué J. Mechanisms of TGF-β signaling from cell membrane to the nucleus. *Cell* 2003;**6**:685–700.

Shime H, Kariya M, Orii A, Momma C, Kanamori T, Fukuhara K, Kusakari T, Tsuruta Y, Takakura K, Nikaido T. Tranilast inhibits the proliferation of uterine leiomyoma cells in vitro through G1 arrest associated with the induction of p21waf1 and p53. *J Clin Endocrinol Metab* 2002;**12**:5610–5617.

Shull MM, Ormsby I, Kier AB, Pawlowskr S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvint D. Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. *Nature* 1992;**6397**: 693–699.

Smith J, Ockleford CD. Laser scanning confocal examination and comparison of nidogen (entactin) with laminin in term human amniochorion. *Placenta* 1994;**1**: 95–106.

Solomon SD, McMurray JJV, Pfeffer MA, Wittes J, Fowler R, Finn P, Anderson WF, Zauber A, Hawk E, Bertagnolli M. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. *N Engl J Med* 2005;**11**:1071–1080.

Sounni NE, Devy L, Hajitou A, Frankenne F, Munaut C, Gilles C, Deroanne C, Thompson EW, Foidart J-M, Noël A. MT1-MMP expression promotes tumor growth and angiogenesis through an up-regulation of vascular endothelial growth factor expression. *FASEB J* 2002;**6**:555–564.

Sounni NE, Noël A. Membrane type-matrix metalloproteinases and tumor progression. *Biochimie* 2005;**3**:329–342.

Steinauer J, Pritts EA, Jackson R, Jacoby AF. Systematic review of mifepristone for the treatment of uterine leiomyomata. *Obstet Gynecol* 2004;**6**:1331.

Stewart EA, Friedman AJ, Peck K, Nowak RA. Relative overexpression of collagen type I and collagen type III messenger ribonucleic acids by uterine leiomyomas during the proliferative phase of the menstrual cycle. *J Clin Endocrinol Metab* 1994;**3**:900–906.

Stovall TG, Ling FW, Henry LC, Woodruff MR. A randomized trial evaluating leuprolide acetate before hysterectomy as treatment for leiomyomas. *Am J Obstet Gynecol* 1991;**6 Pt 1**:1420–1423. ; discussion 1423-5.

Stovall TG, Muneyyirci-Delale O, Summitt RL Jr, Scialli AR. GnRH agonist and iron versus placebo and iron in the anemic patient before surgery for leiomyomas: a randomized controlled trial. The Leuprolide Study Group. *Obstet Gynecol* 1995; **1**:65–71.

Stracke JO, Hutton M, Stewart M, Pendás AM, Smith B, López-Otin C, Murphy G, Knäuper V. Biochemical characterization of the catalytic domain of human matrix metalloproteinase 19. Evidence for a role as a potent basement membrane degrading enzyme. *J Biol Chem* 2000;**20**:14809–14816.

Strand S, Vollmer P, van den Abeelen L, Gottfried D, Alla V, Heid H, Kuball J, Theobald M, Galle PR, Strand D. Cleavage of CD95 by matrix metalloproteinase-7 induces apoptosis resistance in tumour cells. *Oncogene* 2004;**20**:3732–3736.

Sun Q, Weber CR, Sohail A, Bernardo MM, Toth M, Zhao H, Turner JR, Fridman R. MMP25 (MT6-MMP) is highly expressed in human colon cancer, promotes tumor growth, and exhibits unique biochemical properties. *J Biol Chem* 2007;**30**: 21998–22010.

Suo G, Jiang Y, Cowan B, Wang JY. Platelet-derived growth factor C is upregulated in human uterine fibroids and regulates uterine smooth muscle cell growth. *Biol Reprod* 2009;**4**:749–758.

Suzuki M, Raab G, Moses MA, Fernandez CA, Klagsbrun M. Matrix metalloproteinase-3 releases active heparin-binding EGF-like growth factor by cleavage at a specific juxtamembrane site. *J Biol Chem* 1997;**50**:31730–31737.

Syssoev KA, Kulagina NV, Chukhlovin AB, Morozova EB, Totolian AA. Expression of mRNA for chemokines and chemokine receptors in tissues of the myometrium and uterine leiomyoma. *Bull Exp Biol Med* 2008;**1**:84–89.

Tang XM, Dou Q, Zhao Y, McLean F, Davis J, Chegini N. The expression of transforming growth factor-beta s and TGF-β receptor mRNA and protein and the effect of TGF-βs on human myometrial smooth muscle cells in vitro. *Mol Hum Reprod* 1997;**3**:233–240.

Taniguchi Y, Morita I, Kubota T, Murota S, Aso T. Human uterine myometrial smooth muscle cell proliferation and vascular endothelial growth-factor production in response to platelet-derived growth factor. *J Endocrinol* 2001;**1**: 79–86.

Thorne JT, Segal TR, Chang S, Jorge S, Segars JH, Leppert PC. Dynamic reciprocity between cells and their microenvironment in reproduction. *Biol Reprod* 2015;**1**: 25.

Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol* 2002; **5**:349–363.

Toullec A, Gerald D, Despouy G, Bourachot B, Cardon M, Lefort S, Richardson M, Rigaill G, Parrini MC, Lucchesi C. Oxidative stress promotes myofibroblast differentiation and tumour spreading. *EMBO Mol Med* 2010;**6**:211–230.

Tsigkou A, Reis FM, Ciarmela P, Lee MH, Jiang B, Tosti C, Shen FR, Shi Z, Chen YG, Petraglia F. Expression levels of myostatin and matrix metalloproteinase 14 mRNAs in uterine leiomyoma are correlated with dysmenorrhea. *Reprod Sci* 2015;**12**:1597–1602.

Turner RS, Thomas RG, Craft S, Van Dyck CH, Mintzer J, Reynolds BA, Brewer JB, Rissman RA, Raman R, Aisen PS. A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer disease. *Neurology* 2015;**16**:1383–1391.

Velasco G, Cal S, Merlos-Suárez A, Ferrando AA, Alvarez S, Nakano A, Arribas J, López-Otín C. Human MT6-matrix metalloproteinase: identification, progelatinase A activation, and expression in brain tumors. *Cancer Res* 2000;**4**:877–882.

Wang H, Lafdil F, Wang L, Yin S, Feng D, Gao B. Tissue inhibitor of metalloproteinase 1 (TIMP-1) deficiency exacerbates carbon tetrachloride-induced liver injury and fibrosis in mice: involvement of hepatocyte STAT3 in TIMP-1 production. *Cell Biosci* 2011;**1**:14.

Wang J, Ohara N, Wang Z, Chen W, Morikawa A, Sasaki H, DeManno DA, Chwalisz K, Maruo T. A novel selective progesterone receptor modulator asoprisnil (J867) down-regulates the expression of EGF, IGF-I, TGFβ3 and their receptors in cultured uterine leiomyoma cells. *Hum Reprod* 2006;**7**:1869–1877.

Wang T, Zhang X, Obijuru L, Laser J, Aris V, Lee P, Mittal K, Soteropoulos P, Wei JJ. A micro-RNA signature associated with race, tumor size, and target gene activity in human uterine leiomyomas. *Genes Chromosomes Cancer* 2007;**4**:336–347.

Wang Y, Feng G, Wang J, Zhou Y, Liu Y, Shi Y, Zhu Y, Lin W, Xu Y, Li Z. Differential effects of tumor necrosis factor-α on matrix metalloproteinase-2 expression in human myometrial and uterine leiomyoma smooth muscle cells. *Hum Reprod* 2015;**1**:61–70.

Webb AH, Gao BT, Goldsmith ZK, Irvine AS, Saleh N, Lee RP, Lendermon JB, Bheemreddy R, Zhang Q, Brennan RC. Inhibition of MMP-2 and MMP-9 decreases cellular migration, and angiogenesis in in vitro models of retinoblastoma. *BMC cancer* 2017;**1**:434.

Wegienka G. Are uterine leiomyoma a consequence of a chronically inflammatory immune system? *Med Hypotheses* 2012;**2**:226–231.

Werner S, Hubner G, Alzheimer C. Activin: a novel player in tissue repair processes. *Histol Histopathol* 1999;**1**:295–304.

Wight TN. Versican: a versatile extracellular matrix proteoglycan in cell biology. *Curr Opin Cell Biol* 2002;**5**:617–623.

Wijelath ES, Rahman S, Namekata M, Murray J, Nishimura T, Mostafavi-Pour Z, Patel Y, Suda Y, Humphries MJ, Sobel M. Heparin-II domain of fibronectin is a vascular endothelial growth factor-binding domain enhancement of VEGF biological activity by a singular growth factor/matrix protein synergism. *Circ Res* 2006;**8**:853–860.

Williams AR, Critchley HO, Osei J, Ingamells S, Cameron IT, Han C, Chwalisz K. The effects of the selective progesterone receptor modulator asoprisnil on the morphology of uterine tissues after 3 months treatment in patients with symptomatic uterine leiomyomata. *Hum Reprod* 2007;**6**:1696–1704.

Wipff P-J, Rifkin DB, Meister J-J, Hinz B. Myofibroblast contraction activates latent TGF-β1 from the extracellular matrix. *J Cell Biol* 2007;**6**:1311–1323.

Wolanska M, Bankowski E. Fibroblast growth factors (FGF) in human myometrium and uterine leiomyomas in various stages of tumour growth. *Biochimie* 2006;**2**: 141–146.

Wolanska M, Sobolewski K, Bańkowski E, Jaworski S. Matrix metalloproteinases of human leiomyoma in various stages of tumor growth. *Gynecol Obstet Invest* 2004; **1**:14–18.

Wolanska M, Sobolewski K, Drożdżewicz M, Bańkowski E. Extracellular matrix components in uterine leiomyoma and their alteration during the tumour growth. *Mol Cell Biochem* 1998;**1**:145–152.

Wortham NC, Alam NA, Barclay E, Pollard PJ, Wagner BE, Manek S, Elia G, Tomlinson IPM. Aberrant expression of apoptosis proteins and ultrastructural

aberrations in uterine leiomyomas from patients with hereditary leiomyomatosis and renal cell carcinoma. *Fertil Steril* 2006;**4**:961–971.

Wu C-H, Shieh T-M, Wei L-H, Cheng T-F, Chen H-Y, Huang T-C, Wang K-L, Hsia S-M. Resveratrol inhibits proliferation of myometrial and leiomyoma cells and decreases extracellular matrix-associated protein expression. *J Funct Foods* 2016;**23**:241–252.

Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007;**3**:524–529.

Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008;**2**:199–210.

Xing Z, Tremblay GM, Sime PJ, Gauldie J. Overexpression of granulocyte-macrophage colony-stimulating factor induces pulmonary granulation tissue formation and fibrosis by induction of transforming growth factor-β1 and myofibroblast accumulation. *Am J Pathol* 1997;**1**:59–66.

Xu J, Luo X, Chegini N. Differential expression, regulation, and induction of Smads, transforming growth factor-β signal transduction pathway in leiomyoma, and myometrial smooth muscle cells and alteration by gonadotropin-releasing hormone analog. *J Clin Endocrinol Metab* 2003;**3**:1350–1361.

Xu Q, Ohara N, Liu J, Amano M, Sitruk-Ware R, Yoshida S, Maruo T. Progesterone receptor modulator CDB-2914 induces extracellular matrix metalloproteinase inducer in cultured human uterine leiomyoma cells. *Mol Hum Reprod* 2008;**3**:181–191.

Yamaguchi Y, Mann DM, Ruoslahti E. Negative regulation of transforming growth factor-β by the proteoglycan decorin. *Nature* 1990;**6281**:281–284.

Yamashita CM, Dolgonos L, Zemans RL, Young SK, Robertson J, Briones N, Suzuki T, Campbell MN, Gauldie J, Radisky DC. Matrix metalloproteinase 3 is a mediator of pulmonary fibrosis. *Am J Pathol* 2011;**4**:1733–1745.

Yang Q, Mas A, Diamond MP, Al-Hendy A. The mechanism and function of epigenetics in uterine leiomyoma development. *Reprod Sci* 2016;**2**:163–175.

Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell* 1991;**4**:841–848.

Yerushalmi GM, Gilboa Y, Jakobson-Setton A, Tadir Y, Goldchmit C, Katz D, Seidman DS. Vaginal mifepristone for the treatment of symptomatic uterine leiomyomata: an open-label study. *Fertil Steril* 2014;**2**:496–500.

Yin P, Lin Z, Reierstad S, Wu J, Ishikawa H, Marsh EE, Innes J, Cheng Y, Pearson K, Coon JS. Transcription factor KLF11 integrates progesterone receptor signaling and proliferation in uterine leiomyoma cells. *Cancer Res* 2010;**4**:1722–1730.

Yin P, Ono M, Moravek MB, Coon JS, Navarro A, Monsivais D, Dyson MT, Druschitz SA, Malpani SS, Serna VA. Human uterine leiomyoma stem/progenitor cells expressing CD34 and CD49b initiate tumors in vivo. *J Clin Endocrinol Metab* 2015;**4**:E601–E606.

Yoshiji H, Kuriyama S, Miyamoto Y, Thorgeirsson UP, Gomez DE, Kawata M, Yoshii J, Ikenaka Y, Noguchi R, Tsujinoue H. Tissue inhibitor of metalloproteinases-1 promotes liver fibrosis development in a transgenic mouse model. *Hepatology* 2000;**6**:1248–1254.

Yu L, Saile K, Swartz CD, He H, Zheng X, Kissling GE, Di X, Lucas S, Robboy SJ, Dixon D. Differential expression of receptor tyrosine kinases (RTKs) and IGF-I pathway activation in human uterine leiomyomas. *Mol Med* 2008;**5-6**:264–275.

Zbucka M, Miltyk W, Bielawski T, Surazynski A, Palka J, Wolczynski S. Mechanism of collagen biosynthesis up-regulation in cultured leiomyoma cells. *Folia Histochem Cytobiol* 2008;**45**:S181–S185.

Zhang X, Huang S, Guo J, Zhou L, You L, Zhang T, Zhao Y. Insights into the distinct roles of MMP-11 in tumor biology and future therapeutics. *Int J Oncol* 2016;**5**:1783–1793.

Zhao X-H, Laschinger C, Arora P, Szászi K, Kapus A, McCulloch CA. Force activates smooth muscle α-actin promoter activity through the Rho signaling pathway. *J Cell Sci* 2007;**10**:1801–1809.

Zheng L-H, Cai F-F, Ge I, Biskup E, Cheng Z-P. Stromal fibroblast activation and their potential association with uterine fibroids (Review). *Oncol Lett* 2014;**2**:479–486.