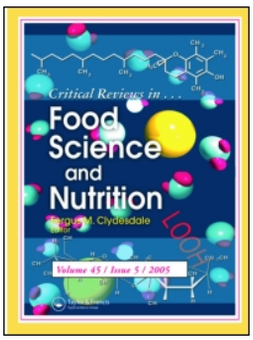

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Molecular targets of dietary phytochemicals for possible prevention and therapy of uterine fibroids: Focus on fibrosis

Md Soriful Islam[a,b], Most Mauluda Akhtar[a,c], James H. Segars[d], Mario Castellucci[a], and Pasquapina Ciarmela[a,e]

[a]Department of Experimental and Clinical Medicine, Faculty of Medicine, Università Politecnica delle Marche, Ancona, Italy; [b]Biotechnology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh; [c]Department of Clinical and Molecular Sciences, Faculty of Medicine, Università Politecnica delle Marche, Ancona, Italy; [d]Howard W. and Georgeanna Seegar Jones Division of Reproductive Sciences, Department of Gynecology and Obstetrics, Johns Hopkins School of Medicine, Baltimore, Maryland, USA; [e]Department of Information Engineering, Università Politecnica delle Marche, Ancona, Italy

ABSTRACT

Uterine fibroids (myomas or leiomyomas) are common benign tumors of reproductive aged women. Fibroids are clinically apparent in 20–50% of women, and cause abnormal uterine bleeding, abdominal pain and discomfort, pregnancy complications and infertility. Unfortunately, limited numbers of medical treatment are available but no effective preventive strategies exist. Moreover, the benefits of medical treatments are tempered by lack of efficacy or serious adverse side effects. Fibrosis has recently been recognized as a key pathological event in leiomyoma development and growth. It is defined by the excessive deposition of extracellular matrix (ECM). ECM plays important role in making bulk structure of leiomyoma, and ECM-rich rigid structure is believed to be a cause of abnormal bleeding and pelvic pain/pressure. Dietary phytochemicals are known to regulate fibrotic process in different biological systems, and being considered as potential tool to manage human health. At present, very few dietary phytochemicals have been studied in uterine leiomyoma, and they are mostly known for their antiproliferative effects. Therefore, in this review, our aim was to introduce some dietary phytochemicals that could target fibrotic processes in leiomyoma. Thus, this review could serve as useful resource to develop antifibrotic drugs for possible prevention and treatment of uterine fibroids.

KEYWORDS

Uterine leiomyoma; growth factors; inflammation; oxidative stress; hypoxia; mechanotransduction

Introduction

Uterine fibroids (also known as leiomyomas, myomas, leiomyomata, and fibromyomas or fibromyomata) are most common type of solid tumor in women of reproductive age (Ciarmela et al., 2011b), with an incidence by age 50 is >80% for black women and nearly 70% for white women (Day Baird et al., 2003). However, they are clinically apparent in approximately 20–50% of women (Buttram Jr and Reiter, 1981). Women may have a single or multiple fibroids, and they can be 10 mm in size or routinely exceed 20 cm (Walker and Stewart, 2005). Fibroids cause a variety of health problems, including abnormal uterine bleeding, abdominal pain and discomfort, pregnancy complications and infertility (Stewart, 2001). Surgery is the main treatment for symptomatic uterine leiomyomas because medical treatments still fail to deliver satisfactory results (Islam et al., 2013b). The annual economic burden of these tumors is estimated to be between $5.9 billion and $34.4 billion in USA (Cardozo et al., 2012). This complex disease process also exerts an enormous burden on health care resources in Australia (Treloar et al., 1999) as well as several European countries, including France, Germany, Italy, Spain, and United Kingdom (Downes et al., 2010).

Taking into account the social costs and associated long-term health problems, several medical treatments have been studied, but no one therapy has emerged as a perfect solution (Islam et al., 2013b). For example, gonadotropin-releasing hormone agonist (GnRHa) is U.S. Food and Drug Administration (FDA)-approved short-term medical therapy, which is concomitantly used with iron therapy for the preoperative hematologic improvement of patients with anemia caused by uterine fibroids. GnRHa reduces leiomyoma and uterine volume (Stovall et al., 1995), but duration of treatment is limited to 3 months or less, because of the risk of irreversible bone loss and osteoporosis caused by the hypoestrogenic state (Friedman et al., 1991; Leather et al., 1993). Ulipristal acetate (formerly known as CDB-2914) is approved in Europe and Canada for preoperative uterine leiomyoma treatment (Melis et al., 2012). This compound causes a reduction of leiomyoma volume and positively improves leiomyoma-related symptoms and quality of life without serious complications (Donnez et al., 2012a, b, 2015). Due to the antiprogestin action, the endometrium may grow and concerns about endometrial growth "unchecked" by progesterone have been raised. Therefore, it is clear that there

CONTACT Pasquapina Ciarmela, Ph.D. p.ciarmela@univpm.it Department of Experimental and Clinical Medicine, Faculty of Medicine, Università Politecnica delle Marche, via Tronto 10/a, 60020 Ancona, Italy; Mario Castellucci, M.D., Ph.D. m.castellucci@univpm.it Department of Experimental and Clinical Medicine, Faculty of Medicine, Università Politecnica delle Marche, via Tronto 10/a, 60020 Ancona, Italy
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is an obvious need for new preventive and therapeutic approaches for the management of uterine leiomyoma.

Fibrosis has recently been recognized as a key pathological event in leiomyoma development and growth (Chegini, 2010; Malik et al., 2010; Fujisawa and Castellot Jr, 2014). Fibrosis may be defined as the accumulation of excess extracellular matrix (ECM) components, including collagens (Wynn, 2008). Since uterine leiomyoma contains an excessive amount of ECM proteins, specifically collagens, fibronectin and versican (Stewart et al., 1994; Arici and Sozen, 2000; Norian et al., 2009), their altered composition strongly supports this notion. ECM plays important role in making bulk structure of leiomyoma, and ECM-rich rigid structure is believed to be a cause of abnormal bleeding and pelvic pain/pressure. A series of events/factors such as, inflammation, oxidative stress, hypoxia, cytokines and chemokines, proinflammatory and profibrotic growth factors, angiogenic growth factors as well as mechanical stress and mechanotransduction have been considered to play important role in the process of fibrosis to drive leiomyoma development and growth (Gentry et al., 2001; Syssoev et al., 2008; Chegini, 2010; Joseph et al., 2010; Norian et al., 2012; Wegienka, 2012; Fletcher et al., 2013, 2014; Hou et al., 2014; Islam et al., 2014b).

Taking into consideration the importance of fibrosis in leiomyoma pathogenesis, there is a great interest in the development of antifibrotic drugs for this tumor. Dietary phytochemicals are nonnutritive compounds with disease-preventive properties, mainly found in fruits, vegetables, cereals, legumes, herbs, spices, nuts, and seeds. They are known to regulate fibrotic process in different biological systems (Islam et al., 2014a). In this context, dietary phytochemicals could play important role in developing new antifibrotic drugs for uterine leiomyoma. At present, very few dietary phytochemicals, including epigallocatechin gallate (Zhang et al., 2010), curcumin (Malik et al., 2009), resveratrol (Catherino et al., 2011), isoliquiritigenin (Kim et al., 2008), and genistein (Moore et al., 2007) have been studied in uterine leiomyoma, and they are mostly known for their antiproliferative effects. Therefore, there is much room for future research in the area of dietary phytochemicals and uterine fibroids. In this review, we introduced 11 dietary phytochemicals (Figure 1) that could target fibrotic process in uterine leiomyoma.

Using dietary phytochemicals to target fibrosis in uterine leiomyoma

Fibrosis is the result of defective repair processes often seen after chronic injury and/or inflammation in a large variety of organs and tissues, such as the kidney, heart, liver, lung, and skin (Wynn, 2007). Pathologic fibrosis is characterized by excessive accumulation of ECM proteins leading to disruption of normal tissue architecture (Wynn, 2008).

One of the key processes in fibrosis is presence of myofibroblasts (Wynn and Ramalingam, 2012). Myofibroblasts play a key role in wound healing and tissue homeostasis, as well as fibrosis. Quiescent cells are activated by tissue injury, inflammation, hypoxia and oxidative stress (Poli, 2000; Higgins et al., 2007; Wynn, 2007; Sen and Roy, 2010; Toullec et al., 2010). Myofibroblast cells are characterized by α-smooth muscle actin (α-SMA) expression, increased secretion of collagen type I and III, and contractility (Wynn, 2008). The cells are also characterized by expression of the ED-A splice variant of fibronectin and the upregulated synthesis of several ECM proteins and growth factors (Serini et al., 1998). The consensus is that myofibroblasts are ultimately responsible for the excessive deposition of ECM in fibrosis.

The potential precursors for myofibroblasts including fibroblasts, mesenchymal stem cells, smooth muscle cells, endothelial cells, and fibrocytes have been reported in various organs (Hinz et al., 2007). In the uterus, myofibroblastic transformation could occur from fibroblasts, stem cells or smooth muscle cells (Ono et al., 2007; Chang et al., 2010; Moore et al., 2010; Holdsworth-Carson et al., 2014; Mas et al., 2014, 2015; Zheng et al., 2014). We have recently identified numerous $CD34^+$ fibroblasts, which are known to give origin to myofibroblasts when CD34 expression is lost (Protic et al., 2015). In addition, Yin and colleagues recently reported the identification of leiomyoma stem/progenitor cells expressing CD34 and CD49b that were able to initiate tumors *in vivo* (Yin et al., 2015). Cytokines, chemokines, growth factors and angiogenic factors, and their complex signaling pathways regulate myofibroblast differentiation (Wynn, 2008, 2007) (Figure 2). After completing the repair and restoring tissue homeostasis, myofibroblast cells disappear through apoptosis (Desmouliere et al., 1995). However, failure of this process and the persistence of cells with a myofibroblastic phenotype are associated with the development of fibrosis (expansion of the extracellular matrix and contraction) (Powell et al., 1999; Tomasek et al., 2002).

Chronic inflammatory reactions are induced by a variety of stimuli including persistent infections, autoimmune reactions, allergic responses, chemical insults, radiation, and tissue injury (Wynn, 2008). In the uterus, there are many causes of chronic low-grade systemic inflammation such as infection, DNA damage, talc use, intrauterine device, caesarean section, male reproductive proteins, obesity, tobacco smoke, some dietary components and medications, injury, stress (work, home, perceived racism), and aging (Wegienka, 2012). In addition, reproductive events such as ovulation, menstruation and implantation create physiological injuries that trigger inflammatory reaction in the uterus. During each menstrual cycle, the functional layer of the endometrium undergoes extensive changes, following complete tissue breakdown and regeneration. The repeated stimulation by reproductive events, mechanical forces, injury, hypoxia and oxidative stress could create a chronic inflammatory state in the uterus (Wegienka, 2012; Leppert et al., 2013). Under a chronic inflammatory state, myofibroblasts cells can produce ECM in an unregulated fashion without going apoptosis, leading to fibrosis (Figure 2). Since the ECM amount and stiffness are critical for the structural and functional integrity of tissues, excess deposition of ECM in fibrosis results in the development and exacerbation of tissue dysfunction (Jourdan-LeSaux et al., 2010). The excessive accumulation of ECM components and cross-linking of these components may further activate resident cells to myofibroblastic transition leading to fibrosis (Desmouliere et al., 2005; Ho et al., 2014) (Figure 2).

Leiomyomas are known to possess a large amount of ECM proteins, primarily collagens, fibronectin and proteoglycans (Stewart et al., 1994; Arici and Sozen, 2000; Norian et al., 2009). An abnormal collagen fibril structure and orientation were found in leiomyomas (Leppert et al., 2004).

Figure 1. Dietary phytochemicals and their chemical structure and sources.

Additionally, relative overexpression of types I, III, and V collagen were found in leiomyomas compared with the normal myometrium (Stewart et al., 1994; Iwahashi and Muragaki, 2011). Recent global gene-profiling experiments have shown that ECM genes encoding collagen proteins were differentially expressed in uterine leiomyomas compared to normal myometrial smooth muscle cells (Tsibris et al., 2002; Catherino et al., 2004; Leppert et al., 2006; Malik et al., 2010). Furthermore, an elevated level of fibronectin expression has been reported in leiomyoma compared with autologous myometrium (Arici and Sozen, 2000). Higher amounts of glycosaminoglycans and versican (a large ECM proteoglycan) were also found in uterine fibroids compared to normal tissues (David et al., 2012). The laminins are heterotrimeric proteins of the ECM that are composed of α-, β-, and γ-sufbunits. Leiomyoma cells demonstrated an elevated amount of total laminins compared to myometrial cells (Malik et al., 2012). ECM accumulation is the most consistent feature of all fibrotic conditions that is regulated by the combined action of MMPs (a class of protein that breaks down the ECM) and TIMPs. Notably, leiomyomas express higher levels of specific MMPs including MMP-2, MMP-11, and MMP-14 (Palmer et al., 1998; Wolanska et al., 2004; Dimitrova et al., 2009; Tsigkou et al., 2015). Additionally, TGF-β3 was reported to decrease production of MMP-2 and MMP-11 (Joseph et al., 2010).

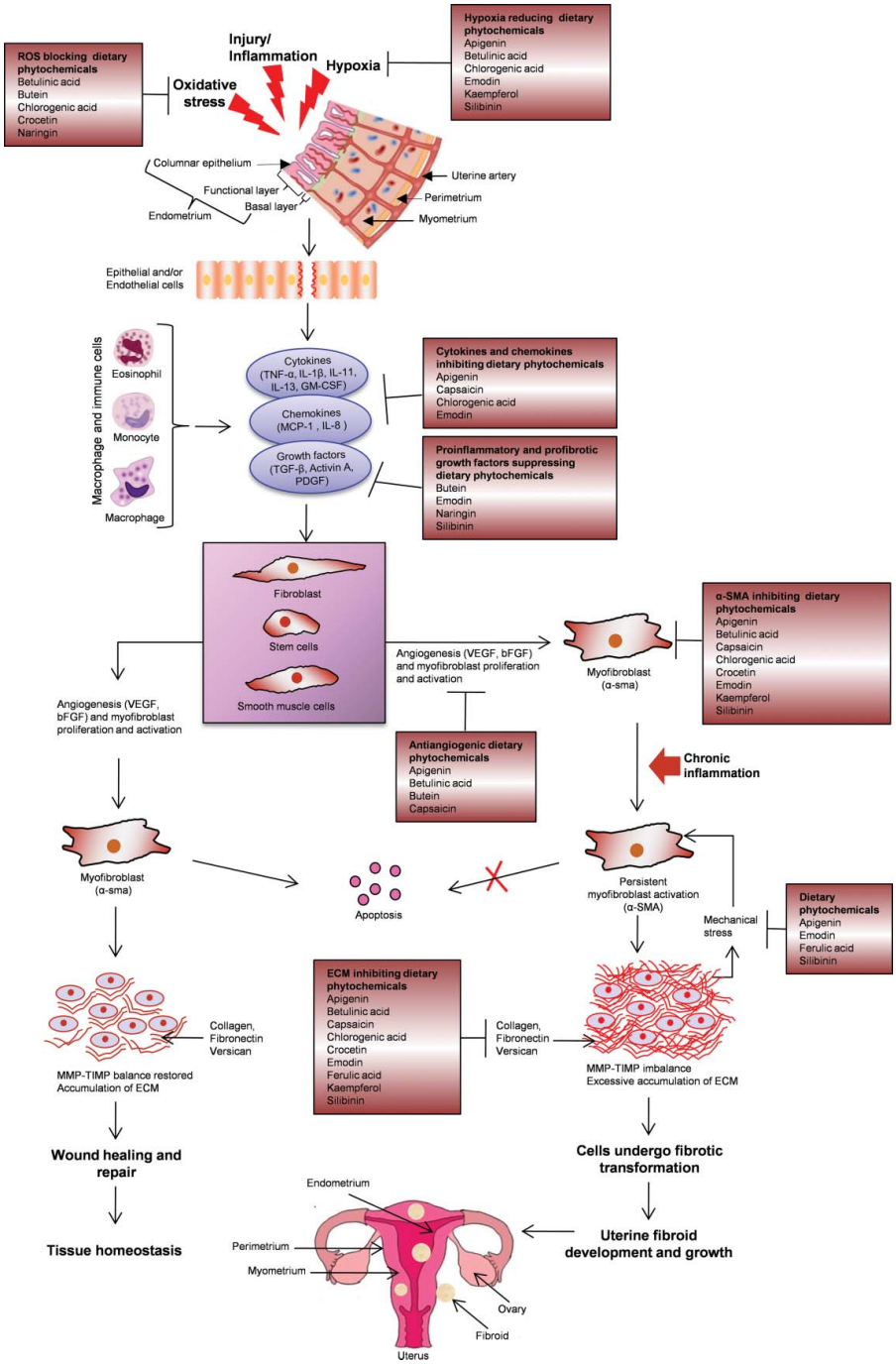

Figure 2. Cellular and molecular pathways underlying fibrosis in uterine leiomyoma development and growth targeted by dietary phytochemicals. Fibrosis is initiated by tissue injury/inflammation, oxidative stress and hypoxia. Cytokines, chemokines and growth factors are produced at the site of injury, and contribute to fibroblast activation and differentiation into myofibroblasts. Angiogenic growth factors also take part in this process. To restore homeostatic condition, myofibroblasts produce ECM components (collagen, fibronectin, versican) and maintain MMP-TIMP balance, consequently wound is healed, and myofibroblasts eliminate by apoptosis. However, under a chronic inflammatory state, myofibroblasts become resistant to elimination by apoptosis, produce excessive amounts of ECM components (collagen, fibronectin, versican), and therefore MMP-TIMP imbalance induces cells to undergo fibrotic transformation. Dietary phytochemicals are shown to interrupt different stages of fibrotic process. ROS, reactive oxygen species; TNF-α, tumor necrosis factor; IL-1β, interleukin-1β; IL-11, interleukin-11; IL-13, interleukin-13; GM-CSF, granulocyte-macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein-1; TGF-β, transforming growth factor β; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; α-sma, α-smooth muscle actin; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; ECM, extracellular matrix.

In order to unify the target of dietary phytochemicals for prevention and treatment of uterine fibroids, we dissected the fibrotic process into several categories such as oxidative stress, hypoxia, cytokines and chemokines, proinflammatory and profibrotic growth factors, angiogenic growth factors as well as mechanical stress and mechanotransduction. Therefore, those factors can be considered as potential targets of antifibrotic drugs (Figure 2).

Oxidative stress

An inadequate antioxidant protection or an excessive production of ROS can alter the cellular oxidative balance creating a condition known as oxidative stress, which is closely related to fibrosis of many organs (Poli, 2000; Manoury et al., 2005; Gabrielli et al., 2012). Oxidative stress has been shown to be an

important factor in the formation of uterine fibroids (; Mesquita et al., 2010; Fletcher et al., 2013, 2014; Santulli et al., 2013). Fibroid cells are reported to have significantly lower levels of antioxidant enzymes, superoxide dismutase and catalase mRNA than normal myometrial cells (Fletcher et al., 2013). In addition, higher concentrations of serum oxidative stress markers (protein carbonyl groups and advanced oxidized protein products) were found in leiomyoma patients than in the control group (Santulli et al., 2013). Fibroid cells are characterized by an unique nicotinamide adenine dinucleotide phosphate oxidase (NOX, a major source of superoxide and subsequent oxidative stress) profile, which promotes a severe prooxidant state that may be responsible for their development (Fletcher et al., 2014). NOX4 has been shown to mediate TGF-β1-induced differentiation of cardiac fibroblasts into myofibroblasts (Cucoranu et al., 2005). A recent study reported that the expression of NOX4 was increased in fibroids compared to myometrial tissues and cells (Fletcher et al., 2014). In addition, NOX2, DUOX1 (dual oxidase 1), and $p67^{phox}$ were higher, while $p22^{phox}$ was lower in fibroid compared to myometrial cells (Fletcher et al., 2014). ROS is formed after exposure to oxidative stress and/or hypoxia that has been implicated in the activation of various signaling molecules (Kamata and Hirata, 1999). Stimulation of primary leiomyoma cells with EGF and PDGF was reported to markedly increase NOX-derived intracellular ROS production that mediates mitogen-activated protein kinase (MAPK)3/MAPK1 (ERK 1/2) activation leading to cell proliferation (Mesquita et al., 2010).

Several dietary phytochemicals such as crocetin (Cai et al., 2009), butein (Szuster-Ciesielska et al., 2013), betulinic acid (Szuster-Ciesielska et al., 2011a), naringin (Chen et al., 2013b), and chlorogenic acid (Li et al., 2015) have been reported to exert antifibrotic effects through regulation of ROS and antioxidant signaling pathways (Figure 2).

Crocetin is an important carotenoid constituent of saffron, a spice and food colorant obtained from the dry stigmas of the plant *Crocus sativus* L. This phytochemical has been shown to exert protective effects against cardiac hypertrophy that was mediated by blocking the ROS-dependent MAPK/ERK 1/2 pathway and GATA binding protein 4 activation (Cai et al., 2009).

Butein is a type of chalcone derivative found in cashews (*Semecarpus anacardium*). This is known as an inhibitor of acetaldehyde-induced activation of liver stellate cells (Szuster-Ciesielska et al., 2011b). A recent report showed that butein inhibits ethanol-induced activation of liver stellate cells partially through inhibition of oxidative stress (Szuster-Ciesielska et al., 2013).

Betulinic acid is a pentacyclic triterpene found in leaves of rosemary (*Rosmarinus officinalis* L.), and fruits of elephant apple (*Dillenia indica* L.). This pentacyclic triterpene was found to attenuate liver stellate cell activation partially by inhibiting ROS (Szuster-Ciesielska et al., 2011a).

Naringin is a flavanone glycoside found in grapefruit (*Citrus paradisi*) and related citrus species. A protective effect of naringin against paraquat-induced acute lung injury and pulmonary fibrosis in mice has been reported (Chen et al., 2013b). This effect was mediated partially through the alleviation of oxidative stress (Chen et al., 2013b). The increased intracellular ROS generation as induced by hypoxia was potently inhibited by chlorogenic acid in pulmonary artery smooth muscle cells (Li et al., 2015).

Hypoxia

Hypoxia is a condition marked by an inadequate oxygen supply to the cells and tissues of the body. It has a role in the development of tissue fibrosis (Higgins et al., 2007). In recent years, a growing number of evidences proved that hypoxia plays an important role in the pathogenesis of uterine fibroids (Mayer et al., 2008; Zhou et al., 2011; Fletcher et al., 2013; Hou et al., 2014; Tadakawa et al., 2015). Fibroid cells have been found to be severely hypoxic compared to the adjacent myometrium (Mayer et al., 2008). Moreover, uterine fibroids have an impaired antioxidant cellular enzymatic system, which may make them more susceptible to hypoxia (Fletcher et al., 2013). It has been hypothesized that oxygen-limited microenvironment protects leiomyoma cells against apoptosis and maintains the state of proliferation (Zhou et al., 2011). HIF-1α is a dimeric protein complex that primarily mediates the transcriptional response to hypoxia. Hypoxia promotes fibrosis via HIF-1α stimulation of epithelial-to-mesenchymal transition (Higgins et al., 2007). The mRNA level of HIF-1α was found to be increased in leiomyomas compared with the adjacent myometrium (Hou et al., 2014). In hypoxia-mimicking conditions, HIF-1α protein was highly expressed in ELT-3 cells and was suppressed by treatment of the anti-diabetic drug metformin (Tadakawa et al., 2015). Thioredoxin-1 is a small redox protein overexpressed in a number of human primary tumors which is known to increase HIF-1α protein expression (Welsh et al., 2002). Expression of thioredoxin-1 was increased in leiomyomas compared with the matched adjacent myometrium (Hou et al., 2014). Endothelin-1 is a vasoactive peptide which is potently secreted from activated endothelial cells in response to hypoxic conditions (Kourembanas et al., 1991). Endothelin-1 has been reported to induce HIF-1α expression in pulmonary artery smooth muscle cells (Li et al., 2012). Of note, circulating levels of endothelin-1 were found to be higher in women with uterine fibroids compared to women without uterine fibroids (Wallace et al., 2014). In addition, endothelin-1 secretion was significantly increased in fibroid explants compared to myometrium explants (Wallace et al., 2014).

Since HIF-1α is induced under hypoxic conditions and plays important role in fibrosis, there is considerable interest to inhibit and/or block this protein by dietary phytochemicals. The ability of several dietary phytochemicals such as apigenin (Fang et al., 2005), betulinic acid (Karna et al., 2010), chlorogenic acid (Li et al., 2015), emodin (Ha et al., 2011), kaempferol (Luo et al., 2009), and silibinin (Garcia-Maceira and Mateo, 2009) to inhibit and/or block HIF-1α accumulation, expression and activity has been reported in different pathological cell types (Figure 2).

Apigenin is an antioxidant plant flavonoid which contains antiinflammatory and anticancer properties. This flavonoid found in wide variety of dietary sources such as parsley, Chinese cabbage, bell pepper, garlic, snake gourd, guava, and

celery. Apigenin has been reported to inhibit protein expression of HIF-1α via the PI3K/AKT/p70S6K1 pathways in human ovarian cancer cells (Fang et al., 2005).

Betulinic acid inhibited collagen biosynthesis in human endometrial adenocarcinoma cells (Karna et al., 2010). This effect was accompanied by a parallel decrease in prolidase activity and expression and HIF-1α in cultured human endometrial adenocarcinoma cells (Karna et al., 2010). HIF-1α is a known regulator of VEGF, and betulinic acid disturbed the binding of HIF-1α and signal transducer and activator of transcription 3 (STAT3) to the VEGF promoter in hypoxic prostate cancer cells (Shin et al., 2011).

Chlorogenic acid (also known as 5-O-caffeoylquinic acid or 3-O-caffeoylquinic acid) is an ester composed of cinnamic acids and quinic acid and it is majorly found in coffee beans. Other dietary sources of chlorogenic acid include apples, pears, stone fruits, berries, artichoke, and aubergines. It was shown that chlorogenic acid suppressed hypoxia-induced HIF-1α protein expression and trans-activation in pulmonary artery smooth muscle cells (Li et al., 2015).

Emodin is a natural anthraquinone compound isolated from the root and rhizomes of Rhubarb (*Rheum palmatum* L.). This natural anthraquinone attenuated the expression of HIF-1α mRNA transcripts in IL-1β and lipopolysaccharide (LPS)-treated synoviocytes under hypoxia (Ha et al., 2011). Kaempferol is a flavonoid commonly found in different edible plants including green tea, broccoli, apples, strawberries and beans. Luo and coworkers demonstrated that HIF-1α protein expression was reduced by kaempferol in ovarian cancer cell lines (Luo et al., 2009). In addition, kaempferol effectively inhibited HIF-1 activity in human hemochromatotic cell lines (Mylonis et al., 2010). Silibinin was also reported to inhibit hypoxia-induced HIF-1α accumulation and HIF-1 transcriptional activity in human cervical and hepatoma cells (Garcia-Maceira and Mateo, 2009). HIF-1α subunit accumulation and VEGF secretion were inhibited by silibinin as well in retinal pigmented epithelial cells (Lin et al., 2013). Furthermore, silibinin inhibited basal and hypoxia induced expression levels of HIF-1α protein in prostate cancer cells (Jung et al., 2009).

Cytokines and chemokines

Recruitment of inflammatory cells to the site of the acute injury is a critical feature of wound healing. However, chronic inflammation is a prolonged pathological condition characterized by mononuclear immune cell infiltration (macrophages, monocytes, lymphocytes, and plasma cells), tissue destruction and fibrosis. Recently, our group demonstrated that higher numbers of macrophages are present inside and in the vicinity of leiomyoma as compared to the more distant surrounding myometrium (Protic et al., 2015). Of note, inflammatory cells in particular macrophages are widely accepted as important regulators of cytokines and growth factors during wound healing (Leibovich and Ross, 1975). Cytokines are low-molecular-weight (10–50 kDa) proteins released by cells of the immune system. Several cytokines have shown biological relevance to leiomyoma pathophysiology. Tumor necrosis factor (TNF)-α is a pleiotropic cytokine produced by many different types of cells, including macrophages (Parameswaran and Patial, 2010). It was found that the protein expression of TNF-α was higher in leiomyoma compared to normal myometrial cells and tissues (Kurachi et al., 2001; Plewka et al., 2013). TNF-α significantly increased 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 increased activin A mRNA expression in both leiomyoma and myometrial cells (Protic et al., 2015). Furthermore, TNF-α has been reported to activate ERK and NF-κB pathways in leiomyoma smooth muscle cells (Wang et al., 2015). Adipose tissues secrete cytokines known as adipokines. Nair and Al-Hendy demonstrated that human adipocytes secreted TNF-α, and coculture with adipocytes increased human uterine leiomyoma cells proliferation through TNF-α (Nair and Al-Hendy, 2011).

IL-1β has been implicated as one of the dominant factors in the development of fibrosis (Kolb et al., 2001; Chaudhuri et al., 2007; Guo et al., 2013). This cytokine was expressed in the acute phase of inflammation, but was also elevated in the later stages of inflammation (Ren and Torres, 2009). The expression of IL-1β mRNA and protein was found to be higher in leiomyomas compared to the control myometrium (Syssoev et al., 2008; Plewka et al., 2013).

IL-11, alone, or through interaction with TGF-β, is considered to play a critical role in the progression of fibrotic disorders (Chakir et al., 2003). It has been shown that leiomyoma smooth muscle cells highly expressed IL-11 compared with myometrial smooth muscle cells (Luo et al., 2005).

IL-13 is a major inducer of fibrosis in many chronic infectious and autoimmune diseases (Fichtner-Feigl et al., 2005; Liu et al., 2012). IL-13 has been reported to induce lung fibrosis through TGF-β1 production (Fichtner-Feigl et al., 2005). Ding and colleagues demonstrated the IL-13 expression in leiomyoma, and discovered that exposure of leiomyoma smooth muscle cells to IL-13 increased expression of TGF-β and TGF-βRII in leiomyoma smooth muscle cells, suggesting a direct and/or indirect regulatory function of IL-13 in the progression of tissue fibrosis in leiomyoma (Ding et al., 2004a).

The overexpression of GM-CSF is known to induce pulmonary granulation tissue formation and fibrosis by induction of TGF-β1 and myofibroblast accumulation (Xing et al., 1997). It was reported that GM-CSF increased its own production, the expression of TGF-β1 mRNA, and the cell-associated TGF-β1 protein content in both myometrial and leiomyoma cells (Chegini et al., 1999).

Chemokines are a family of small cytokines, induced during an immune response to recruit cells of the immune system to a site of infection. They are known to be critical mediators of diverse biological processes including development, angiogenesis, hematopoiesis and fibrosis. The expression profiles of many chemokines and their receptors have been reported in leiomyomas and matched myometrium (Sozen et al., 1998; Senturk et al., 2001; Syssoev et al., 2008). MCP-1 is known to play an important role in the inflammatory response of blood monocytes and tissue macrophages. Sozen and colleagues found this chemokine at higher levels in myometrium compared with leiomyoma (Sozen et al., 1998).

Eotaxins are responsible for eosinophil recruitment. Chemokine (C-C motif) receptor (CCR) 3 is a specific receptor for

these compounds. The eotaxin and CCR3 mRNA were found to be increased in myometrial tissue compared to leiomyoma (Syssoev et al., 2008). Monocytes and T lymphocytes expressing CCR1 and CCR5 are the targets for macrophage inflammatory protein (MIP)-1α and MIP-1β. It was reported that the content of MIP-1α, MIP-1β, and CCR5 mRNA were higher in myometrium compared to leiomyoma tissue (Syssoev et al., 2008).

IL-8 binds to chemokine (C-X-C motif) receptor 1 (CXCR1) and CXCR2, which contributes to chemotaxis and activation of neutrophilic granulocytes. Expression of mRNA transcripts for IL-8 and CXCR1 in myometrial tissues surrounding uterine leiomyoma was 2-fold higher than in tumor tissue (Senturk et al., 2001). Myometrial IL-8 expression was reported to increase by stretch and the inflammatory cytokines in a MAPK- and NF-κB-dependent manner (Sooranna et al., 2005; Hua et al., 2012; Chuang and Khorram, 2014).

NF-κB is a key transcriptional regulator of many genes associated with inflammation, fibrosis and tumorigenesis (Elsharkawy and Mann, 2007; DiDonato et al., 2012). Functionally, NF-κB is sequestered in the cytoplasm in association with IkBs. Phosphorylation of IκB by IkB kinase and proteasome-dependent degradation results in NF-κB dissociation and nuclear translocation (DiDonato et al., 2012). Once in the nucleus, NF-κB regulates a plethora of genes coding for cytokines and chemokines (Ghosh and Karin, 2002). A higher expression of NF-κB proteins has been observed in leiomyomas compared to myometrium (Plewka et al., 2013). Curcumin, an inexpensive dietary supplement found in turmeric, was reported to inhibit NF-κB protein expression in leiomyoma cells (Malik et al., 2009). The involvement of NF-κB dependent inflammatory pathway in leiomyoma cells was further documented by the observation that EGCG significantly decreased the expression of NF-κB-dependent pathway genes such as PCNA (proliferating cell nuclear antigen), CDK4 (cyclin-dependent kinase 4), and Bcl 2 (B-cell lymphoma 2) as well as increase the expression of the proapoptotic BAX (B-cell lymphoma 2 associated X) in a dose-dependent manner (Zhang et al., 2010). An essential role for NF-κB in the regulation of chemokine IL-8 in human myometrial and leiomyoma cells has been reported (Khanjani et al., 2012; Chuang and Khorram, 2014). Chuang and Khorram demonstrated that miR-200c decreased nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, α (IkBα) phosphorylation which caused NF-κB p65 cytoplasmic sequestration, and thereby, repressed IL8 expression in leiomyoma smooth muscle cells (Chuang and Khorram, 2014).

Several dietary phytochemicals such as apigenin (Nicholas et al., 2007), capsaicin (Park et al., 2004), chlorogenic acid (Shi et al., 2009), and emodin (Chen et al., 2009) with antiinflammatory effect have all been reported in *in vitro* and *in vivo* conditions to affect key pathways involved in leiomyoma formation (Figure 2).

For instance, apigenin was shown to suppress LPS-induced proinflammatory mediators such as IL-1β, IL-8, and TNF in human monocytes and mouse macrophages (Nicholas et al., 2007). This phytochemical likewise suppressed phorbol 12-myristate 13-acetate plus A23187-induced expression of TNF-α, IL-8, IL-6, and GM-CSF by decreasing the intracellular Ca^{2+} level and inhibiting NF-κB activation in human mast cells (Kang et al., 2011). Furthermore, apigenin inhibited TNF-α-induced cell proliferation and PGE synthesis by inactivating NF-κB in endometriotic stromal cells (Suou et al., 2011). In addition, apigenin suppressed TNF-α-induced expression of CCL2/MCP-1 and CXCL1/KC (Funakoshi-Tago et al., 2011) and markedly inhibited acute carrageenan-induced paw edema in mice (Funakoshi-Tago et al., 2011). Moreover, apigenin was reported to inhibit the expression of IL-6 and IL-8 in di-(2-ethylhexyl) phthalate stimulated human umbilical vein endothelial cells (HUVECs) (Wang et al., 2012).

Capsaicin, active component of chili peppers, has been reported to suppress the production of TNF-α by acting as an agonist for PPARγ in LPS-stimulated murine macrophages (Park et al., 2004). In addition, capsaicin exhibited an antiinflammatory property by the inhibition of PGE2 and nitric oxide (NO) production in peritoneal macrophages via NF-κB inactivation (Kim et al., 2003).

Chlorogenic acid inhibited carbon tetrachloride 4 $(CCl)_4$-induced liver fibrosis in rats, which was partly mediated by inhibition of inflammatory mediators (Shi et al., 2009). The hepatic mRNA expression and serum levels of TNF-α, IL-6, and IL-1β were found significantly increased in CCl_4-treated rats and attenuated by co-treatment with chlorogenic acid (Shi et al., 2009). Chlorogenic acid suppressed CCl_4-induced NF-κB activation as well (Shi et al., 2009).

Emodin may attenuate the development of pulmonary fibrosis because of its ability to downregulate the elevated levels of IL-4 and IL-13 in bronchoalveolar lavage fluid of bleomycin treated mice (Chen et al., 2009). Emodin was also reported to suppress IL-1β induced mesangial cells proliferation and ECM (fibronectin and collagen) production via inhibiting P38 MAPK pathway (Wang et al., 2007b). MMP-1 is thought to be one of the key enzymes acting in fibrolysis, a process closely related to tissue remodeling. It was reported that emodin significantly inhibited TNF-α -induced MMP-1 gene expression through the inhibition of the activator protein −1 (AP-1) signaling pathway (Lee et al., 2006).

Proinflammatory and profibrotic growth factors

Growth factors are polypeptides or proteins that are secreted by a number of cell types, may play a critical role in inflammation, fibrosis and proliferation (Branton and Kopp, 1999; Werner and Alzheimer, 2006; Ciarmela et al., 2011b; Islam et al., 2013a). Activin-A, TGF-β, and PDGF have been proposed as key proinflammatory and profibrotic mediators in the process of fibrosis following leiomyoma development and growth (Liang et al., 2006; Chegini, 2010; Islam et al., 2014b; Protic et al., 2015).

Activin is a pleiotropic growth factor that belongs to the TGF-β superfamily of growth factors. The role of activin-A in inflammation is well documented (Jones et al., 2007). Increased circulating levels of activin-A were reported in several inflammatory conditions including, septicemia (Michel et al., 2003), inflammatory bowel disease (Hubner et al., 1997), asthma (Karagiannidis et al., 2006), and rheumatoid arthritis (Ota et al., 2003). Many inflammatory cell types such as, monocytes (Eramaa et al., 1992), macrophages (Ebert et al., 2007), dendritic cells (Scutera et al., 2008), and endothelial cells (Wilson et al., 2006) were reported to be source of activin-A. Activin-A production can be up-regulated with TNF-α and IL-1 in the human marrow stromal cells and monocytes (Shao et al.,

1992), as well as thrombin and angiotensin in rat aortic smooth muscle cells (Pawlowski et al., 1997). Furthermore, activin-A accumulation may occur during following positive feedback process: TGF-β stimulates myofibroblasts to produce activin-A, and activin-A can stimulate the production of TGF-β by lung and renal fibroblasts (Aoki et al., 2005; Karagiannidis et al., 2006). The role of activin-A in inflammation is further supported by the finding that the administration of follistatin, an activin-A antagonist, inhibits the onset of the inflammatory cytokine cascade (Jones et al., 2007). The role of activin-A in the driving of myofibroblast differentiation leading to fibrosis has been reported in many fibrotic conditions such as, skin injury (Fumagalli et al., 2007), liver fibrosis (Wada et al., 2004), pancreas fibrosis (Ohnishi et al., 2003), kidney fibrosis (Yndestad et al., 2004), and pulmonary fibrosis (Matsuse et al., 1996). For example, HSCs were reported to be a major source of activin-A in fibrotic rat livers (Sugiyama et al., 1998). HSCs are activated by activin-A, resulting in elevated expression of α-SMA and collagen in these cells, finally culminating in their transformation into myofibroblastic phenotype (Wada et al., 2004).

Higher expression levels of activin-A were found in leiomyoma compared with adjacent myometrial explants (Ciarmela et al., 2011a; Tsigkou et al., 2015). Recently, our group found that TNF-α increased the mRNA expression levels of activin-A in both human primary myometrial and leiomyoma cells (Protic et al., 2015). Of note, activin-A can increase expression of ECM proteins such as, collagen 1A1, fibronectin and versican, and activate the Smad 2/3 signaling pathway in leiomyoma cells, demonstrating its possible role in the driving of myofibroblast differentiation during the process of fibrosis (Islam et al., 2014b).

TGF-β is the prototype of a family of secreted polypeptide growth factors. It has three isoforms: TGF-β1, TGF-β2 and TGF-β3. In addition to activin-A, TGF-β is often associated with chronic phases of inflammatory diseases such as, Crohn's disease (di Mola et al., 1999), asthma (Duvernelle et al., 2003), and rheumatoid arthritis (Taketazu et al., 1994). TGF-β attracts monocytes and other leukocytes to the inflammation site, thus participating in the initial step of chronic inflammation. This mediator plays an active role in the processes of wound healing and fibrosis (Leask and Abraham, 2004). Once the fibroblasts are activated, TGF-β promotes mitogenesis and upregulates the synthesis of numerous components of ECM, leading to fibrosis (Zheng et al., 2014). Involvement of TGF-β in several fibrotic disorders such as, skin injury (Beanes et al., 2003), hepatic fibrosis (Gressner et al., 2002), pancreatic fibrosis (Van Laethem et al., 1996), renal fibrosis (Chung and Lan, 2013) and pulmonary fibrosis (Kang et al., 2007) have been reported.

TGF-β and its receptors are expressed in human myometrium (Chegini et al., 1994; Tang et al., 1997) and leiomyomas (Dou et al., 1996; Chegini et al., 2003; Xu et al., 2003; Ding et al., 2004b). TGF-β1 has been reported to increase inflammatory and profibrotic mediators such as, IL-11 (Luo et al., 2005), connective tissue growth factor (CTGF) (Luo et al., 2006), c-fos, c-jun, and plasminogen activator inhibitor 1 (PAI-1) (Ding et al., 2004b), fibromodulin (a collagen-binding protein) (Levens et al., 2005) in myometrial and leiomyoma smooth muscle cells. TGF-β1 also induced a more prominent increase in the DNA synthesis of leiomyoma cells compared with myometrial cells at low concentrations, which was partly mediated through the up-regulation of PDGF secretion (Arici and Sozen, 2003). Furthermore, TGF-β1 has been reported to activate Smad 2/3 (Xu et al., 2003) and ERK 1/2 (Ding et al., 2004b) signaling in both myometrial and leiomyoma smooth muscle cells. In addition to TGF-β1, TGF-β3 also increased mRNA expression of ECM components such as, collagen 1A1, CTGF (Joseph et al., 2010), and versican V0 (Norian et al., 2009) in myometrial and leiomyoma cells. TGF-β3 also induced fibronectin mRNA expression in leiomyoma cells and directly stimulated myometrial and leiomyoma cell proliferation in cultures (Arici and Sozen, 2000). The results above strongly support the conclusion that myometrial cells undergo fibrotic transformation under the influence of TGF-β1 and/or TGF-β3.

PDGF is a dimeric growth factor. The biologically active PDGF protein forms disulphide-bonded dimers, including four homodimers PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD, and one heterodimer, PDGF-AB. PDGF has a role in the myofibroblast development during fibrosis (Heldin and Westermark, 1999; Chen et al., 2011; LeBleu and Kalluri, 2011; Singh et al., 2014). PDGF causes neutrophil, macrophage, fibroblast, and smooth muscle cell proliferation and migration into the wound site (Heldin and Westermark, 1999). It is known that PDGF promotes pericyte recruitment to vessels. Chen and colleagues demonstrated that PDGF receptor signaling can activate pericyte-myofibroblast transition in obstructive and post-ischemic kidney fibrosis (Chen et al., 2011). The potential role of PDGF in myofibroblast transition was further documented by the observation that blockade of PDGF receptor signaling reduced myofibroblast number and attenuated renal fibrosis (LeBleu and Kalluri, 2011). In addition, blockade of PDGF-B and TGF-β signaling inhibited myofibroblast development from both bone marrow-derived and keratocyte-derived precursor cells *in vivo* (Singh et al., 2014). Furthermore, PGDF stimulates fibroblasts to contract collagen matrices and differentiate into myofibroblasts *in vitro* (Jinnin et al., 2005; Rhee and Grinnell, 2006).

PDGF and PDGF-R expression have been found in both normal myometrium and leiomyoma cells (Boehm et al., 1990; Mangrulkar et al., 1995); with elevated protein levels of PDGF-AA, PDGF-BB and PDGF-CC (Liang et al., 2006; Suo et al., 2009) and their receptors (Liang et al., 2006; Yu et al., 2008) in leiomyoma compared to myometrium. In leiomyoma and myometrial cells, PDGF has been reported to increase collagen α1 (I) mRNA expression (Liang et al., 2006). Furthermore, PDGF can activate ERK 1/2 signaling in leiomyoma smooth muscle cells leading to cell proliferation (Mesquita et al., 2010). PDGF also interacts with other growth factors such as TGF-β and EGF to enhance proliferation (Fayed et al., 1989; Wolanska and Bankowski, 2007). These results suggest that PDGF may be involved in the process of myofibroblastic transformation during leiomyoma growth.

A number of antifibrotic potential dietary phytochemicals such as, apigenin (Wójcik et al., 2013), betulinic acid (Wan et al., 2012), butein (Szuster-Ciesielska et al., 2013), capsaicin (Bitencourt et al., 2012), chlorogenic acid (Shi et al., 2009), crocetin (Song et al., 2013), emodin (Chen

et al., 2009), ferulic acid (Xu et al., 2015), kaempferol (Gong et al., 2013), naringin (Chen et al., 2013b), and silibinin (Chen et al., 2013c) have been reported to inhibit growth factors, ECM component and associated signaling pathways (Figure 2). It is helpful to consider how these compounds might be used to target uterine fibroid growth.

For instance, apigenin was reported to attenuate TGF-β1-induced fibroblast-to-myofibroblast transition in cultures of primary human bronchial fibroblasts (Wójcik et al., 2013). In human lung myofibroblasts, apigenin blocked thymidine incorporation and reduced basal and TGF-β-stimulated levels of α-SMA and α1(I) collagen mRNA (Ricupero et al., 2001).

Betulinic acid exerts an antifibrotic effect by regulating ECM production, profibrotic cytokine, epithelial-to-mesenchymal transition, and intercellular signaling pathways. Betulinic acid inhibited collagen biosynthesis in human endometrial adenocarcinoma cells (Karna and Palka, 2009). In addition, betulinic acid attenuated liver stellate cell activation partially by inhibiting phosphorylation of Smad3, and activation of TGF-β1 transduction signaling (Szuster-Ciesielska et al., 2011a). Betulinic acid was also reported to exert antifibrotic effect by attenuating the expression of α-SMA and TIMP-1, increasing the levels of MMP-13 (Wan et al., 2012). Furthermore, betulinic acid suppressed neutrophil gelatinase-associated lipocalin-induced epithelial-to-mesenchymal transition in melanoma (Gheorgheosu et al., 2013).

Butein has been reported to be an inhibitor of acetaldehyde-induced activation of liver stellate cells (Szuster-Ciesielska et al., 2011b), and also inhibited myofibroblastic differentiation of rat HSCs (Woo et al., 2003) and liver fibrosis induced by CCl_4 (Lee et al., 2003). A recent report showed that butein inhibited ethanol-induced activation of liver stellate cells through inhibition of TGF-β, p38 MAPK, and JNK signaling pathways (Szuster-Ciesielska et al., 2013). Similarly, capsaicin, the active component of chili peppers, has also been shown to induce de-differentiation of activated HSCs (Bitencourt et al., 2012). Chlorogenic acid inhibited CCl_4-induced liver fibrosis in rats, accompanied by reduced mRNA expression of collagen I and collagen III, as well as protein expression of α-SMA (Shi et al., 2009).

An antifibrotic effect of crocetin has been reported in scleroderma fibroblasts and in bleomycin-induced sclerotic mice (Song et al., 2013). In *in vitro*, crocetin decreased the expression of α-SMA and the levels of mRNA for collagen1A1, collagen3A1 and MMP-1, while crocetin increased TIMP-1 mRNA levels in both systemic scleroderma and normal fibroblasts. *In vivo*, crocetin alleviated the thickening of the dermis and lung fibrosis, and decreased collagen1A1 mRNA levels in the skin and lung of the bleomycin-induced sclerotic mice (Song et al., 2013).

Emodin, an anthraquinone derivative, has been reported to have salutary effects on bleomycin-induced pulmonary fibrosis in mice, which was accompanied by decreased collagen production by fibroblasts (Chen et al., 2009). Emodin also prevented hepatic fibrosis by inhibiting α-SMA expression (Zhan et al., 2001) and HSC activation (Dong et al., 2009). In addition, emodin exhibited antifibrotic effect on pancreatic fibrosis, which was partly related to reduced expression of collagen and TGF-β1 (Wang et al., 2007a). Furthermore, emodin suppressed elevated glucose-induced cell proliferation and fibronectin expression in rat mesangial cells by inhibiting a p38 MAPK pathway involving the CREB (cAMP response element-binding protein), PPARγ and CTGF, suggesting a potential antifibrotic role of emodin in diabetic nephropathy (Li et al., 2009).

Ferulic acid is a ubiquitous polyphenolic compound in plant kingdom, found especially in artichokes, auberginess and maize bran. This polyphenolic compound was reported to significantly decrease the levels of collagen and TIMPs, while positively modulating expression of MMPs in alcohol and heated polyunsaturated fatty acid induced liver toxicity in male albino Wistar rats (Rukkumani et al., 2012). A recent study reported that ferulic acid suppressed activation of HSCs through ERK 1/2 and Smad signaling pathways (Xu et al., 2015). In addition, ferulic acid significantly inhibited both viability and activation of HSC-T6 cells, and dramatically decreased the mRNA and protein level of α1(I) collagen and fibronectin expression (Xu et al., 2015).

Kaempferol has been reported to inhibit TGF-β-induced epithelial-mesenchymal transition by reversing E-cadherin expression and retarding the induction of N-cadherin and α-SMA (Gong et al., 2013). In addition, oral administration of kaempferol suppressed collagen deposition, epithelial excrescency and goblet hyperplasia in the lung of ovalbumin-challenged mice (Gong et al., 2013).

The protective effects of naringin against paraquat-induced acute lung injury and pulmonary fibrosis in mice have been reported (Chen et al., 2013b). This effect was mediated primarily through downregulation of expression of TGF-β1, as well as modulation of expression and ratios of MMP-9 and TIMP-1 (Chen et al., 2013b).

Silibinin (also known as silybin) is a flavonolignan compound found in artichokes. This flavonolignan compound has been reported to attenuate cardiac hypertrophy and fibrosis by partially blocking TGF-β1/Smad signaling pathways (Ai et al., 2010). Chen and colleagues reported that silibinin inhibited TGF-β1-induced expression of α-SMA, vimentin, collagen contraction, CTGF, type 1 collagen in human tenon fibroblasts through downregulation of TGF-βR-related Smad signaling pathways (Chen et al., 2013c). Silibinin also inhibited myofibroblast transdifferentiation in human tenon fibroblasts and reduced fibrosis in a rabbit trabeculectomy model (Chen et al., 2013c). A recent study indicated that silibinin has the potential to prevent fibrotic skin changes by inducing the downregulation of type I collagen expression in human skin fibroblasts, which was partly mediated by the inhibition of the Smad2/3-dependent signaling pathway (Cho et al., 2013).

Angiogenic growth factors

Angiogenesis, a key step in tumor growth, is a biological process by which new blood vessels develop from the endothelium of a pre-existing vasculature (Folkman, 1990). In normal circumstances, the formation of new blood vessels occurs during wound healing, organ regeneration and in the female reproductive system during ovulation, menstruation, and the formation of the placenta (Hoeben et al., 2004). The sequential steps in angiogenesis include: (1) release of proteases from "activated" endothelial cells with subsequent degradation of the basement

membrane surrounding the existing vessels; (2) migration of endothelial cells into the interstitial space; (3) endothelial cell proliferation, and differentiation into mature blood vessels; and (4) with lumen formation, generation of new basement membrane with the recruitment of pericytes, and finally blood flow (Bussolino et al., 1997). Newly formed blood vessels supply required nutrients and oxygen to tumors, dispose of metabolic waste products, and generate paracrine stimuli allowing for tumor expansion (Folkman, 1990; Risau, 1990; Carmeliet, 2003). Tumors can promote blood vessel growth (angiogenesis) by secreting growth factors (such as VEGF, bFGF, PDGF, TGF-β, etc.) that stimulate endothelial migration, proliferation, proteolytic activity, and capillary morphogenesis (Risau, 1990). The role of angiogenesis in leiomyoma growth is supported by the observation that several angiogenic growth factors such as, EGF, HB-EGF, VEGF, bFGF, PDGF, TGF-β and adrenomedullin are differentially expressed in leiomyomas compared with normal myometrium (Tal and Segars, 2014). Notably, common treatments for fibroids, such as GnRH agonists, have been shown to work, at least in part, via antiangiogenic mechanisms (Tal and Segars, 2014). Among the studied angiogenic growth factors, VEGF and bFGF are thought to be more important for sustaining leiomyoma growth.

VEGF, a major contributor to angiogenesis, increases the number of capillaries in a given network (Ferrara and Davis-Smyth, 1997). VEGF mRNA (Harrison-Woolrych et al., 1995) and VEGF-A protein (Gentry et al., 2001) have been detected in myometrium and leiomyoma. The VEGF receptors, VEGFR-1 and VEGFR-2 are also expressed in myometrium (Brown et al., 1997) and leiomyomas (Sanci et al., 2011). Gentry and coworkers demonstrated a higher expression of VEGF-A antigen in leiomyomas compared to the adjacent myometrium (Gentry et al., 2001). It was shown that pretreatment of leiomyoma xenografts with VEGF was required for the continued growth of leiomyoma tissue *in vivo* (Hassan et al., 2008). Of note, GnRHa and ulipristal acetate treatment decreased leiomyoma vascularization and was associated with the reduction of VEGF expression (De Falco et al., 2006; Xu et al., 2006). In addition, ulipristal acetate has also been shown to be able to block the activin-A-induced VEGF-A mRNA expression in myometrial and leiomyoma cultured cells (Ciarmela et al., 2014).

bFGF is a potent inducer of angiogenesis that induces the promotion of endothelial cell proliferation and the physical organization of endothelial cells into tube-like structures. Protein and mRNA expression of both bFGF and its receptors FGFR-1 and FGFR-2 have been identified in leiomyoma and myometrium (Pekonen et al., 1993; Mangrulkar et al., 1995; Anania et al., 1997; Dixon et al., 2000). A higher expression of bFGF was observed in leiomyoma compared with myometrium (Mangrulkar et al., 1995; Wolanska and Bankowski, 2006). In addition, FGFR-1 and FGFR-2 expression have been found to be increased in leiomyoma compared with adjacent myometrium (Wolanska and Bankowski, 2006; Yu et al., 2008). A distinctive characteristic of uterine fibroids is the presence of an increased amount of ECM compared to normal myometrium. bFGF has been found to be primarily bound to the ECM of both myometrium and fibroids, whereas fibroids showed much stronger staining for bFGF (Mangrulkar et al., 1995; Dixon et al., 2000). It is known that the growth factor content of the ECM determines endothelial cell angiogenic responses (Dye et al., 2004) supporting the notion that increased bFGF content of the ECM found in leiomyomas may play an important role in angiogenesis within fibroids. Notably, GnRHa treatment of women with fibroids has been shown to decrease protein expression of bFGF and total number of vessels in the leiomyomas (Di Lieto et al., 2003, 2005).

Apigenin (Fang et al., 2005), betulinic acid (Kwon et al., 2002), butein (Moon et al., 2010), and capsaicin (Min et al., 2004) have been reported to inhibit angiogenic factors (Figure 2) as wells as angiogenic process such as proliferation, migration, and tube formation of endothelial cells.

Apigenin, a flavonoid, has been reported to inhibit tube formation *in vitro* by endothelial cells (Fang et al., 2005). In addition, apigenin inhibited expression of HIF-1α and VEGF via the PI3K/AKT/p70S6K1 pathways (Fang et al., 2005).

Betulinic acid, a pentacyclic triterpene found in the bark of several species of plants widespread throughout the tropics, was reported to potently inhibit bFGF-induced invasion and tube formation of bovine aortic endothelial cells (Kwon et al., 2002). Betulinic acid also showed antiangiogenic effect in LNCaP prostate cancer cells by decreasing expression of VEGF (Chintharlapalli et al., 2007). Shin and colleagues reported that betulinic acid exerts its antiangiogenic activity by disturbing the binding of HIF-1α and STAT3 to the VEGF promoter in hypoxic PC-3 human prostate cancer cells (Shin et al., 2013). The final step of collagen degradation is mediated by prolidase which may play a role in angiogenesis. Karna and coresearchers reported that betulinic acid may exert antiangiogenic potential by inhibition of prolidase, HIF-1α and VEGF expressions, and also collagen biosynthesis in cultured endometrial adenocarcinoma cells (Karna et al., 2010).

Butein is a plant polyphenol that is classified as a chalcone, has been reported to inhibit invasion and angiogenesis in prostate cancer cells by attenuating VEGF activities through the suppression of NF-κB activity (Moon et al., 2010). Butein also inhibited serum- and vascular VEGF induced cell proliferation, migration, and tube formation of human endothelial progenitor cells (Yeh and Wang, 2013) and markedly abrogated VEGF-induced vessels sprouting from aortic rings and suppresses microvessel formation in the Matrigel implant assay *in vivo* (Yeh and Wang, 2013).

Capsaicin showed a potent antiangiogenic activity *in vitro* and *in vivo* (Min et al., 2004). This compound inhibited VEGF-induced proliferation, migration, and tube formation of endothelial cells *in vitro* (Min et al., 2004) and markedly inhibited tumor- or VEGF-induced new blood vessel formation in chick chorioallantoic membrane and Matrigel plug assays *in vivo* (Min et al., 2004).

Mechanical stress and mechanotransduction

Mechanical stress is a well documented stimulus of muscle hypertrophy. Several studies have addressed tissue biomechanics and mechanical signaling in myometrial and leiomyoma cells (Rogers et al., 2008; Malik et al., 2012; Norian et al., 2012). Due to excessive accumulation of ECM components and cross-linking of these components, alterations in tissue stiffness are common features of fibrosis. It was reported that uterine

Table 1. Dietary phytochemicals under clinical trials.

Phytochemicals	ClinicalTrials.gov identifier	Conditions	Phase	Present status
Apigenin	NCT00609310	Colorectal cancer	Phase II	Suspended
Betulinic acid	NCT00346502	Dysplastic nevus syndrome	Phase I & Phase II	Suspended
Capsaicin	NCT02037464	Prostate cancer	Phase II	Not yet recruiting
	NCT00003610	Head and neck cancer, radiation toxicity	Phase III	Completed
	NCT01478607	Painful diabetic peripheral neuropathy	Phase III	Completed
Chlorogenic acid	NCT02728349	Glioblastoma	Phase I	Recruiting
	NCT02621060	Impaired glucose tolerance	Phase II	Recruiting
	NCT02136342	Advanced cancer	Phase I	Terminated
Kaempferol	NCT02425436	Intrauterine Growth Restriction (IUGR)	Phase II	Completed
Naringin	NCT01272167	Post-menopausal status		Completed
Silibinin	NCT00684268	Hepatitis C	Phase II	Completed
	NCT00915200	Diabetic nephropathies, Proteinuria, Oxidative stress	Phase II	Completed
	NCT02633696	Healthy volunteers	Phase I	Completed
	NCT01129570	Advanced hepatocellular carcinoma	Phase I	Completed
	NCT01003236	Diabetic nephropathy	Phase II	Completed
	NCT01049178	Atopic asthma	Phase II & III	Withdrawn

leiomyoma is significantly stiffer than matched myometrium (Rogers et al., 2008). The increased stiffness is accompanied by alteration of the ECM, cell shape, and cytoskeleton in leiomyoma compared with myometrium (Rogers et al., 2008). Increased ECM stiffness can modify mechanical stress on cells that lead to the activation of Rho-dependent signaling (Paszek et al., 2005; Rogers et al., 2008). RhoA belongs to the Rho family of small GTPases that function as molecular switches to cycle between the inactive GDP-bound and active GTP-bound state (Wettschureck and Offermanns, 2002). Leiomyoma cells demonstrated an increased expression of active RhoA compared to myometrial cells (Norian et al., 2012). In addition, the levels of A kinase anchor protein 13 (AKAP13, a protein that is known to activate Rho), was increased in leiomyoma compared to myometrium (Rogers et al., 2008).

Mechanotransduction is the capability of cells to convert mechanical stress into cell signaling (Alenghat and Ingber, 2002). Mechanotransduction occurs at sites of focal adhesions, which are plasma membrane structures that serve as a nexus between the ECM and the contractile actin cytoskeleton
(Geiger et al., 2001). Mechanical signals are transmitted from the ECM scaffold via transmembrane receptors, called integrins (α and β). Integrin activation by ECM induces focal adhesion kinase (FAK) autophosphorylation and that Src binds to and is activated by FAK to mediate downstream signaling pathways such as PI3K and ERK (Guo and Giancotti, 2004). In leiomyoma cells, the levels of integrin β1 were found to be increased compared with their normal counterparts (Malik et al., 2012; Chen et al., 2013a). The phosphorylation of FAK and its downstream kinase, ERK, was evident in leiomyomal cells (Chen et al., 2013a), and the levels of FAK and ERK phosphorylation were decreased in integrin β1 knockdown leiomyomal cells (Chen et al., 2013a). In addition, disruption of ECM-integrin interaction by the small protein, disintegrin, inhibited cyclin D1 expression and cell proliferation (Chen et al., 2013a). The findings of increased integrin β1 and mechanical signaling molecules support the overall fibrotic nature of this disease.

Furthermore, it has been proposed that myofibroblast differentiation depend on a combination of mechanical tension with TGF-β activity and specialized matrix molecules (ED-A splice variant of fibronectin) (Hinz, 2006; Wipff et al., 2007). TGF-β is induced by tension, which, in turn, activates collagen synthesis via the classic pathways (Lindahl et al., 2002). Induction of ED-A fibronectin, requires presence of TGF-β together with mechanical tension, indicating that complex paracrine and autocrine networks are involved in regulating myofibroblast formation (Serini et al., 1998; Tomasek et al., 2002).

Dietary phytochemicals such as apigenin (Franzen et al., 2009), emodin (Kim et al., 2005), ferulic acid (Xu et al., 2015), and silibinin (Deep et al., 2014) have been reported to inhibit integrin, FAK and its downstream kinase, ERK (Figure 2).

Apigenin, a common plant flavonoid, has been shown to possess antitumor properties. Franzen and colleagues demonstrated that apigenin inhibited motility and invasion of prostate carcinoma cells, disrupted actin cytoskeleton organization (Franzen et al., 2009) and decreased activation of FAK and Src, as well as phosphorylation of Src substrates FAK Y576/577 and Y925 (Franzen et al., 2009). Apigenin has also been reported to inhibit expression of FAK and migration and invasion of human ovarian cancer A2780 cells (Hu et al., 2008).

The ability of emodin, an anthraquinone derivative, to inhibit phosphorylation of FAK, ERK 1/2 and AKT/PKB has been reported in glioma cells (Kim et al., 2005).

Ferulic acid is a polyphenolic compound commonly present in cereals that has been reported to suppress activation of hepatic stellate cells (Xu et al., 2015). It was reported that ferulic acid inhibited ERK1/2 phosphorylation concomitant with a significant decrease in the expression of FAK (Xu et al., 2015).

Silibinin, the major bioactive constituent present in silymarin which is mainly isolated from milk thistle, was reported to decrease the fibronectin-induced cell proliferation and motility of human prostate carcinoma PC3 cells via targeting integrin signaling (Deep et al., 2014). Silibinin also decreased fibronectin-induced expression of integrins (α5, αV, β1 and β3), actin-remodeling (FAK, Src, GTPases), and cell survival (survivin and AKT) related signaling molecules in PC3 cells (Deep et al., 2014).

Conclusions

Fibrosis is well studied in many organs (such as kidney, heart, liver, lung, etc.), but this process is still under investigation in uterine leiomyoma. However, accumulating evidences suggest that oxidative stress, hypoxia, cytokines and chemokines, proinflammatory and profibrotic growth factors, angiogenic growth factors as well as mechanical stress and mechanotransduction are involved in this process to drive leiomyoma development and growth. Taking into consideration the critical role of fibrosis in leiomyoma pathogenesis, this review introduces 11 dietary phytochemicals (apigenin, betulinic acid, butein, capsaicin, chlorogenic acid, crocetin, emodin, ferulic acid, kaempferol, naringin, and silibinin) (Figure 1) that have shown antifibrotic effect in different biological systems. These dietary phytochemicals may regulate fibrotic processes in uterine fibroids development and growth through: (I) neutralizing oxidative stress by decreasing ROS production; (II) inhibiting and/or blocking hypoxia induced HIF-1α accumulation; (III) cytokines (TNF-α, IL-1β, IL-11, IL-13, GM-CSF) and chemokines (MCP-1, IL-8) as well as NF-κB transcription factor; (IV) proinflammatory and profibrotic growth factors (TGF-β, Activin A, PDGF), ECM components (collagen, fibronectin) and associated signaling pathways (Smad 2/3, ERK 1/2), as well as (V) mechanical stress and mechanotransduction (Integrin, FAK, AKT, ERK). Therefore, future research may include those dietary phytochemicals (not limited to 11) to check their ability for modulating fibrotic process in uterine fibroids to develop new preventive and/or therapeutic option for this condition. Additionally, there are ongoing or completed clinical trials (clinicaltrials.gov) of several dietary phytochemicals, including apigenin, betulinic acid, capsaicin, chlorogenic acid, kaempferol, naringin, and silibinin (Table 1) in diverse pathological and healthy conditions, which supports the possible use of those dietary phytochemicals in the prevention and/or treatment of uterine leiomyoma.

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