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Non-hormonal mediators of uterine fibroid growth.

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Abstract

Purpose of the review—Uterine fibroids (UFs) are the most common benign neoplasms of the female reproductive tract and one of the major public health concerns. Although most women with UFs are asymptomatic, over 30% of them will present with varying symptoms.

This review focuses on the role of non-hormonal mediators and pathways in UF biology. Furthermore, it provides data regarding the most recent findings in the field of compounds which use those non-hormonal pathways in the medical therapy of UFs.

Recent findings—Complex signaling pathway alterations are crucial for UF development. The topic of the pathophysiology of UFs focuses mostly on steroids and other hormones. However, other very important pathways exist which are independent of hormones. Some of the most important pathways which are non-hormonal, but in some cases still hormone-depended, include growth factors, cytokines and inflammation, Smad proteins, wingless type/β-catenin and others.

Summary—Much more is known about hormonal than about non-hormonal signaling in UFs. Growth factors, early life exposure and inflammation are key factors in UF biology. Numerous agents depend on those pathways and may find their place in the current and future therapy of UFs.

Keywords

uterine fibroid; leiomyoma; pathophysiology; biology; non-hormonal; mediator; therapy; pharmacology

Introduction

Uterine fibroids (UFs) are the most common benign neoplasms of the female reproductive tract and a major public health concern in reproductive age women. The overall incidence of those lesions is hard to determine since there are only few well performed studies. However,

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Conflicts of interest

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in some populations it may even reach 70% of women [1]. Although most women with UFs are asymptomatic, over 30% of them will present with various symptoms. UFs cause significant morbidity and may compromise daily activities, relationships or workplace performance [2, 3]. The overall quality of life among women with UFs is seriously impacted and it decreases with an increase in the number and severity of symptoms. Lesions that cause these kind of symptoms may often require a medical intervention [4, 5].

Despite their prevalence, not much is known about the reasons they occur and there are still more questions than answers [2, 6]. According to a recent systematic review by Stewart et al. (2017) the most important risk factors for UF are race (e.g. African American), older age, premenopausal state, hypertension, family history of UFs, nulliparity, time since last birth, and different food additives as well as soy [1]. Obesity and vitamin D deficiency are also key factors [7].

UFs are made of abnormal smooth uterine muscle cells and fibroblasts surrounded by extensive amount of extracellular matrix (ECM) including collagens, fibronectin, laminins, and proteoglycans [8, 9]. One of the most important things about UFs is their tendency to grow which, depending on the location, may cause clinical symptoms. Some authors indicated that large UFs grow slowly, while very small ones grow rapidly but with a higher chance of disappearance [10]. The UF life cycle may be divided into two steps – transformation and tumor formation [8, 11]. The transformation of normal uterine smooth muscle cells into abnormal ones occurs mostly through mutations in the mediator complex subunit 12 *(MED12)* gene [12] and the high mobility group AT-hook 2 *(HMGA2)* gene [8, 13]. Stem cells transform and grow into UFs mostly under the influence of hormones and the growth of the tumors occurs via massive cell expansion and ECM accumulation [6, 8, 9].

UF cells are dependent on stimulation, especially by estrogen and progesterone. Available data suggest that progesterone plays a more important role in their development [8, 14]. However, despite the data concerning steroids, we cannot disregard other important pathways involved in this process, as the hormones are not the only factor responsible for UF development [15, 16]. Data about those pathways and mediators are still incomplete. However, recent research has provided answers to several difficult questions. Signals forwarded by various growth factors, Smad protein, phosphatidylinositol-3-kinase and the mammalian target of rapamycin – PI3K/AKT/mTOR pathway, mitogen-activated protein kinases/extracellular signal-regulated kinases – MAPK/ERK pathway, wingless-type (Wnt)/β-catenin and retinoic acid pathways are involved in UF growth and proliferation indicating that they could serve as targets for possible therapies [9, 17, 18].

This review focuses on the role of non-hormonal mediators and pathways in UF biology. Furthermore, it provides data regarding the most recent findings in the field of compounds which use those non-hormonal pathways in the medical therapy of UFs.

Review of pathways

Non-hormonal pathways in uterine fibroids—Complex signaling pathway alterations are crucial for UF development, but the exact underlying biology of UFs is unclear. The topic of the pathophysiology of UFs focuses mostly on steroids and other hormones [8, 14,

19]. However, other very important pathways exist which are not only hormone-dependent. This review will add information about hormones only when necessary and we will focus on other pathways. These include:

- growth factors,
- cytokines and inflammation,
- Smad proteins,
- Wnt/β-catenin,
- peroxisome proliferator-activated receptor (PPAR),
- retinoic acid (RA).

The important finding for better understanding is that many of those pathways converge, link or overlap and incorporate input from several different directions [17].

Growth factors and related signaling—One of the main mechanisms of UF growth consists in the overexpression of cytokine-related genes and the increase of growth factor levels directly in the tumor [18, 20]. Their influence results in the activation of intracellular signal transduction pathways that modulate cell life. Growth factors are proteins that regulate numerous aspects of cellular function, including survival, proliferation, migration and differentiation. The term ‘growth factor’ is sometimes used by scientists interchangeably with the term ‘cytokine’ [20, 21]. Some growth factors that play a role in UF biology have been known for years. These are (in alphabetical order): epidermal growth factor (EGF) [22], fibroblast growth factor (FGF) [23], insulin-like growth factor (IGF) [24], platelet-derived growth factor (PDGF) [25], neuronal growth factor (NGF) [26], transforming growth factor (TGF) [18], and vascular endothelial growth factor (VEGF) [27]. Data concerning TGF-β in the pathophysiology of UFs are the most abundant, as it is believed to be one of the major links in both hormonal and non-hormonal pathways [18]. TGF-β3 was shown to be a key player in UFs with its role in cell proliferation and the deposition of the ECM [9, 18], which may serve as a reservoir of pro-fibrotic growth factors and as a stabilizer of their duration of signaling [9]. TGF-β-derived pathways are mostly connected with progesterone. However, many stages do not depend on steroids and they will be described in the present review.

Growth factors (e.g. TGF-β) bind to receptor tyrosine kinases (RTKs) causing the dimerization and phosphorylation of the receptor and activation of several pathways [17, 28]. RTKs are a big family of cell surface receptors that act with growth factors, hormones, neurotrophic factors and other extracellular signaling molecules [29]. According to Yu et al. ‘cross talk’ occurs between estrogens and RTK signaling pathways and estrogen upregulates RTKs in UFs [28]. Abnormalities that affect RTK signaling might lead to cell transformation and even to the onset of a malignant process [29]. RTK activation influences the activation of PI3K kinase and this in turn regulates mTOR, which was found to be upregulated in UFs both in the human and animal model [30, 31]. It also has an effect on forkhead box O transcription factors (FoxO) or Bcl-2 family proteins and different kinases, transcription factors and many other molecules to facilitate cell survival and cell cycle entry [17, 29].

The Ras/Raf/MEK/ERK signaling pathway is the second one that may be influenced by growth factors due to RTK interactions [32]. This pathway plays a major role in different physiological and pathological processes. Its activation triggers a cascade of which leads to the phosphorylation of target proteins in the nucleus and cytoplasm [17]. Briefly, activated Ras activates Raf kinases, then it phosphorylates MEK protein that activates ERK and several other transcription factors [17, 33]. It is critical that the bidirectional interaction between steroid signaling and the Ras/Raf/MEK/ERK pathway occurs, but growth factors may modulate the response to steroids through the effect of ERK on the transcriptional activity of steroid receptors [17]. As this pathway is both hormonal and non-hormonal, the growth factors produced without the influence of hormones might have their effect on its modulation. Since RTK-derived pathways are so important in the context of tumor biology current data suggest that they might be a target of several drugs [29]. Therefore, the extensive research in the area of RTKs, the MAP kinase and PI3K/AKT pathways is an unmet need in new medical therapies in UFs.

Cytokines, inflammation and their role in signaling in UFs—Inflammation is the response of tissues to harmful stimuli and various cytokines play an important role in its regulation [34]. Inflammation and its derived microenvironment have a pivotal influence on the risk of various courses of disease. In case of UFs, inflammation switches mostly to immunosuppression due to tumor evasion from anti-tumor immune response. However, in many cases this pathway is still unknown and hard to describe in detail [35]. Early life and prenatal exposure to different stimuli increase the risk of diseases in later life, due to the inflammation-derived reprogramming of the epigenome. It is frequently one the main causes of UF occurrence [35]. Paracrine signaling plays an important role in the cellular transformation of the myometrium. Cytokines may be responsible for UF-associated symptoms, such as pain or infertility, but nowadays more authors are focusing on their role in UF growth [16, 36].

Inflammation influences UF proliferation and growth via various mechanisms that include the alteration of the ECM. Andaloussi et al. (2017) emphasized that some cross talk occurred between hormones and inflammation in the pathogenesis of UFs. It is mostly related to their possible connection with UF-associated genetic defects including the most important *MED12* gene mutation [37]. Moreover, chronic inflammation seems to be even more harmful to the uterus. As found by Orciani et al. (2018) the progenitor cells of UFs secreted significantly higher levels of cytokines with chronic inflammation rather than acute inflammation [15]. It may support the data that obesity [7] or hypertension [1] could also strongly influence the risk factors for UF occurrence. Immune response and DNA damage regulation with different compounds is also an important chapter in UF biology. The lack of different compounds, e.g. vitamin D in diet, is one of the causes of inflammation, genomic instability and cell proliferation induction [38]. Conversely, if some substances like phytoestrogens [39, 40] are in excess, the compounds become involved in DNA damage, immune response, and tumor occurrence and growth [39].

Steroids play a key role in these processes. Estrogen also plays a powerful role in immune response [38]. According to Murphy et al. (2010) those hormones were able to regulate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activity and the

production of pro-inflammatory cytokines through various mechanisms [41]. Progesterone also exerts an important anti-inflammatory effect, as it inhibits the activation of NF-κB and slows down the upregulation of cyclooxygenase 2 (COX-2) [42]. With all those mechanisms UFs have a special ability to avoid immune response. It may be connected with the fact that the uterus has a specific immunological role which is necessary in the maintenance of pregnancy to term [43].

NF-κB, which is a transcription factor involved in cell survival, proliferation and inflammatory response, is one of the pathways in UF-related inflammation. The second highly important pathway is connected with tumor necrosis factor α (TNF-α). It causes the activation of MAPKs and Tank-binding kinase 1 [37]. Numerous cytokines are involved in UF biology, but TNF-α appears to be one of the most important myometrium-associated cytokines, especially in relation to the clinical symptoms [16, 44]. Kurachi et al. (2001) found that TNF-α obtained in the proliferative phase was higher than in the secretory phase in UFs and that progesterone influenced the decrease of TNF-α expression [45]. TNF-α concentration was also found to be elevated in women with clinically symptomatic UFs, which suggests the presence of an inflammatory state in women with UFs [36]. Current data support the hypothesis that activin A exerts a potent fibrotic effect in UFs, and TNF-α was found to increase activin A messenger RNA (mRNA) expression in UFs [46]. It completes a complex cycle where TNF-α plays a role as activin A upregulator, activin A affects progesterone, which, in turn, inhibits TNF-α [44, 47]. Finally, TNF-α has a potent influence on ERKs, which play a role in integrating external signals from different mitogens and are a part of the Ras/Raf/MEK/ERK signal transduction cascade [44, 48]. There is a great deal of research into the impact of inflammation on UFs and their development and growth with an eye to finding clinical interventions.

Smad protein signaling—Smads are a family of structurally similar proteins that are the signal transducers for the TGF-β superfamily. They transmit signals from several cell membrane receptors to the nucleus [49]. Smads are believed to be extremely important for regulating cell biology. They play a role in proteasome-mediated degradation as they interact with ubiquitin ligases [50]. Smad ubiquitination-related factors have been implicated in their degradation. As suggested by available data, the degradation is implicated in the turnover of Smad mutants that may be connected with different lesions and may contribute to the growth and progression [51, 52]. The Smad pathways are complicated. Upon ligand binding to Smad-coupled receptors they heterotrimerize in several steps and the resulting complex moves to the nucleus to act as a transcription factor [53]. The TGF-β pathway includes Smads that play a role in the regulation of cellular proliferation and ECM formation at the transcriptional level [17, 54]. TGF-β modulates the expression of anti-fibrotic microRNA (miRNA) to maintain the pro-fibrotic functions of its signaling pathway. Smads have major connections with miRNAs that are of great importance in UFs. According to available data *miR-21* promotes excessive ECM formation by stopping the Smad7 protein [55]. It was also found by Liang et al. (2014) also found that overexpression of that the overexpression of *miR-26a* inhibited the nuclear translocation of active Smads which might cause collagen deposition [56]. Smad3 and Smad4 were overexpressed in UFs in comparison with healthy myometrium. This overexpression changed when gonadotropin-releasing hormone (GnRH)

agonist treatment was implemented [57]. The findings clearly indicate that this pathway is of high importance and may be the target of various anti-UF agents, including hormones.

Wingless type/β-catenin signaling—The Wnt signaling is a group of signal transduction pathways which are fundamental mechanisms that direct cell proliferation, polarity and fate determination. A critical and the most studied pathway in this signaling is the canonical Wnt, which plays a major role in the control of key developmental gene expression programs [58]. The Wnt pathways include various glycolipoproteins that pass signals into cells through their surface receptors [58]. The surface receptors, called Frizzled receptors, after connecting with their ligands, cause receptor activation and the phosphorylation of the cytoplasmic proteins, which leads to decreased β-catenin degradation and its accumulation in the cytoplasm. Its several steps trigger the activation of various transcription factors [17]. Accumulated cytoplasmic β-catenin reacts with chromatin and the family of T-cell transcription factor proteins. It changes the expression of a large number of genes and alters key cellular functions which might lead to tumorigenesis [8].

More evidence is available on Wnt/β-catenin and UF occurrence and growth. Research started around 2005, when it was found that the Wnt-derived gene is overexpressed in UFs [59]. Later, Tanwar et al. (2009) discovered that the constitutive expression of activated β-catenin in the animal model led to mesenchymal UF-like tumor development [60]. Mäkinen et al. (2011) found one of the major genetic bases of UFs. The authors examined several UFs via exome sequencing and identified tumor-specific mutations in the mediator complex subunit 12 *(MED12)* gene in about 70% of patients [12]. Stem cells derived from UF tissue, but not from healthy myometrium, were the *MED12* gene mutations carriers. It suggests that a genetic hit transforms a myometrial stem cell into an abnormal one which will constitute the beginning of a UF tumor [8]. Mutations in the *MED12* gene may lead to alterations in the interactions between this gene and β-catenin, leading to the inhibition of β-catenin transactivation in response to Wnt signaling [61].

According to other authors *MED12* mutations overexpress Wnt4, a Wnt protein family member. Along with estrogen activation, it may lead to the occurrence of UF-like lesions in murine models [62]. A recent study by Ali et al. (2020) showed that estradiol induced β-catenin nuclear translocation and, consequently, its responsive genes in both the myometrium and UFs [63]. In 2013 Ono et al. demonstrated that the paracrine activation of the Wnt/β-catenin pathway in UF stem cells promoted tumor growth [64]. It was then suggested that the canonical Wnt pathway may be a potential therapeutic target for the treatment of UFs [65] or it may function as a biomarker [13]. Further research revealed that *MED12* silencing reduced the proliferation of UF cells and it was mediated by this canonical pathway [66]. El Andaloussi et al. (2020) demonstrated that *MED12* mutation presented a potential to transform cells by dysregulating Wnt4/β-catenin which affected mTOR signaling and might cause autophagy abrogation, cell proliferation, and tumorigenesis [67]. Furthermore, very recent interesting data by Harada et al. (2018) suggested that the inhibition of the canonical pathway signaling under hypoxia and starvation may initiate adipocyte differentiation and metaplasia in UFs, which might trigger its change into lipoleiomyoma [68].

Additionally, the Wnt/β-catenin pathway leads to the increased levels of TGF-β3 which is considered to be one of the key factors in the pathophysiology of UFs [18]. Some of those findings were supported with studies that used anti-UF agents which caused the attenuation of this pathway by reducing TGF-β3 signal and protein expression, resulting in a reduction in TGF-β canonical signaling [69]. Corachán et al. (2019) has recently found that the pleiotropic effect of vitamin D might also influence this pathway, as it causes cell growth arrest and Wnt/β-catenin pathway inhibition, but not via apoptosis regulation [70]. Further research in this field is of great importance as current data indicate that this pathway might be one of the best targets for UF therapy.

Peroxisome proliferator-activated receptor signaling—PPARs are a group of nuclear receptor proteins that function as transcription factors [71]. PPARs play various roles, most of them connected with the regulation of cell development, differentiation, metabolism and potential tumor formation [71, 72]. There are three types of those receptors: alpha, delta/beta and gamma [71]. They act as different heterodimers in association with an activator complex that binds to DNA sequence, which leads to the transactivation and transrepression of various genes [71].

There is growing evidence for the role of PPARγ signaling in UF development and growth, especially concerning the gamma family. PPARγ levels were higher in UFs in comparison with healthy myometrium [73]. PPARγ signaling may lead to the inhibition of UF growth through modulating steroid signaling as the stimulation of PPARγ causes the inhibition of estrogen-derived gene expression [74]. Finally, it was found that some agents might influence UF cells through those receptors [75]. Therefore, it was concluded that this pathway might be a potential target to be modulated by new therapeutic compounds [17]. Curcumin, a natural agent, was found to act as a PPARγ ligand and inhibited UF cell proliferation [76]. Further research also showed that it extensively regulated other key pathways, such as MAPK and TGF-β/Smad, which may suggest that the pathways do not work on their own, but are a complicated network of molecular action [77]. However, data about PPARs and UFs are still scarce and there is not much research in the field. We hope that the forthcoming years will bring more high quality studies in this area.

Retinoic acid signaling—All-trans-retinoic acid, commonly known as retinoic acid (RA), is an active metabolite of vitamin A that mediates the functions of this vitamin required for growth and development [78]. RA may be produced in humans in two sequential oxidation steps [79]. RA receptors act as heterodimers with retinoid X receptors (RXRs) [80]. Moreover, RXRs also act as heterodimers for several different nuclear receptors, including the vitamin D receptor (VDR), PPARs and the thyroid hormone receptors [81]. These connections should be treated as a kind of evidence that aberrations in the RA pathways may play a role in UF development and growth, especially considering their relations with PPARs or the VDR.

Available data showed that UF cells responded to RA [82]. Various researchers found that the gene expression of some proteins, enzymes and receptors varied in the RA molecular pathway in UFs in comparison with healthy myometrium [83–85]. Further research revealed

that different levels of RXRs might be the cause of abnormal transcriptional activity in UFs [17].

Modulating RA signaling may be a potential target in therapy. Pharmacological studies support the findings about the influence of RA signaling in UFs. RXR ligands were found to reduce the size of UFs in the animal model [86]. Gilden et al. (2012) found that an RA metabolic blocking agent, liarozole, inhibited ECM protein production in immortalized UF cells, whereas normal myometrial cells demonstrated no significant alteration in ECM regulation [87].

Pharmacological treatment of uterine fibroids – hormonal vs non-hormonal agents

Increasing knowledge of the role of hormonal and non-hormonal signaling links in the pathophysiology of UFs may present an opportunity to understand this difficult topic and constitute a step towards the use of specific signal transduction inhibitors for the treatment and prevention of UFs.

Treatment for UFs depends on the patient's symptoms and her personal goals [88, 89]. Definitive treatment such as surgery may be important in patients wishing to conceive quickly whereas medical interventions may be indicated in patients whose primary concerns are bleeding and/or pain [90, 91]. And undeniable problem associated with UFs is that pharmacological treatment options are limited [4, 92].

Poor understanding and the lack of data about the precise molecular mechanism in UFs is the reason for the limitation of medical treatment. However, the benefits of medical treatment, such as lower costs or better acceptance in patients, are tempered with average efficacy [93]. Recent publications have shed light on new interesting hormonal compounds, such as selective progesterone receptor modulators (SPRMs): ulipristal acetate (UPA) [92], vilaprisan (VPR) [94] and new oral gonadotropin-releasing hormone (GnRH) analogs, like elagolix [95, 96], relugolix [97] or linzagolix [98]. However, UPA might cause negative effect on the liver [99] and VPR studies were discontinued due to the negative findings in its long-term use in animals [94]. Therefore, the field of pharmacological treatment in UFs is still open.

Non-hormonal interventions, both natural and synthetic, would be appealing to patients if effective [100, 101]. Current data concerning various compounds that use non-hormonal pathways in UFs are presented in Table 1.

The majority of substances presented in Table 1 are of natural origin, which suggests that various substances make up our environment and it is advisable to take a closer look to find new solutions that might be useful in UF treatment [120, 121]. In case of UFs substances using non-hormonal pathways are a new issue which seems to be an attractive option for future research. Therefore, our review appears to contain even more necessary information for researchers interested in the topic. Obviously, it seems rather unlikely that preparations using non-hormonal pathways might completely replace hormonal treatment. It is mostly due to the fact that hormonal determinants are unquestionably of key importance in UFs, while non-hormonal ones are rather additional pathways which interlock with them. Another

issue is related to the early stages of knowledge concerning those associations and the paucity of studies. Currently, the proposal of combined therapies, e.g. hormonal and non-hormonal, is an attractive option for further research. Such a treatment might aim at obtaining synergism with one compound assisting the other, making the clinical effect considerably more beneficial [122]. The use of several substances for a specific indication is not as common in UF treatment as in, for example, cardiology [123, 124], but in our viewpoint it seems to be highly rational. Preliminary studies are available is this area, e.g. in case of vitamin D and UPA [125] or vitamin D and EGCG [126], However, the data are scarce for the time being. Seemingly, it is important to suggest that other researchers, both clinicians and laboratory ones, should seek new effective combinations of substances to obtain a more beneficial clinical effect and the optimal symptom reduction in patients. We hope that research will provide simple, non-invasive, and effective treatments.

Conclusion

UF growth involves a complex network of various signaling pathways. Increasing data about the role of hormonal signaling in UFs stand in disproportion to the level of knowledge concerning non-hormonal signaling. However, as UFs are still a great mystery, research concerning non-hormonal pathways in those tumors may present an opportunity to understand this difficult issue and be a step towards the use of specific signal transduction inhibitors for treatment and prevention.

Numerous non-hormonal agents, both natural and synthetic ones, may find their place in the therapy of UFs. Since all the pathways are linked in some manner, we face important therapeutic implications. Finding non-hormonal therapeutic targets will help to serve a group of patients who would prefer an alternative to surgical intervention

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Key points

- Growth factors are important hormonal and non-hormonal mediators in uterine fibroid biology.
- Early life exposure and inflammation are key factors in uterine fibroid development and growth.
- The Wnt/β-catenin pathway is of considerable importance in uterine fibroids being a subject of extensive research these years.
- Modulating retinoic acid signaling may be a potential target in uterine fibroid therapy.
- Oral gonadotropin-releasing hormone analogs and non-hormonal compounds are the future in uterine fibroid therapy.

Table 1.

Examples of compounds using non-hormonal pathways in medical treatment of uterine fibroids.

Natural compound	Author	Example of non-hormonal molecular effect in uterine fibroids
Berberine	Wu et al. 2015 [102] Chuang et al. 2017 [103]	Inflammation inhibition – reduced COX-2 expression.
Curcumin	Malik et al. 2009 [104] Tsuiji et al. 2011 [76] Yu et al. 2019 [77]	ERK 1, ERK 2, and nuclear factor kappa B (NF-κB) pathway inhibition. Cell proliferation inhibition, PPARγ ligand. MAPK, PPAR and TGF-β/Smad regulation.
Epigallocatechin gallate (EGCG)	Zhang et al. 2010 [105]	The upregulation of the TGF-β and stress pathways, the inhibition of the NF-κB-dependent inflammatory pathway. Decrease in the expression of proliferating cell nuclear antigen (PCNA), cyclin-dependent kinase 4 and B-cell lymphoma 2 (Bcl-2). Apoptosis increase with the expression of the Bcl-2-associated X (Bax).
Fucoidan	Chen et al. 2018 [106]	The downregulation of fibronectin, vimentin, α-smooth muscle actin (α-SMA) and collagen 1A1 protein levels in TGFβ3-induced UF cells. The abrogation of TGFβ3-induced levels of Smad2 and ERK1/2, as well as β-catenin translocation into the nucleus.
Halofuginone	Grudzien et al. 2010 [107] Koohestani et al. 2016 [108]	The reduction of collagen type I and collagen type III mRNA levels, as well as of the profibrotic factor TGFbeta1 mRNA levels. Tumor volume reduction through an increase in apoptosis and a reduction in cell proliferation, but no significant changes at the transcript level of the expression of *TGFβ1, TGFβ3* and transforming growth factor β receptors I and II (*TGFβ-R1, TGFβ-R2*) genes.
Indole-3-carbinol	Greco et al. 2020 [109]	mRNA levels of collagen 1A1 decrease in UF cells. Pathway connected with signaling in the ECM.
Isoliquiritigenin	Lin et al. 2019 [110]	Co-treatment with estradiol and isoliquiritigenin inhibited ERK1/2 activation. p38 and c-Jun N-terminal kinase (JNK) activation enhancement. The downregulation of the expression of ECM-associated proteins and matrix metalloproteinases (MMPs).
Locostatin	Janjusevic et al. 2016 [111]	The activation of the MAPK signaling pathway and ERK phosphorylation. Reduced glycogen synthase kinase 3 β (GSK3β) expression, which even with the above-mentioned activation led to the reduction of the proliferation and migration of UF cells.
Pifenidone	Lee et al. 1998 [112]	The inhibition of the proliferation of UF cells and suppression of the mRNA levels of collagen type I and collagen type III.
Resveratrol	Wu et al. 2016 [113] Chen et al. 2019 [114]	Cell cycle arrest and apoptosis regulation. ECM-related protein expression reduction. MMP-2 and MMP-9 expression inhibition. PCNA, fibronectin protein expression decrease, Bax and Bcl-2 *in vivo*. Fibronectin, collagen type 1 and α-SMA protein expression. β-catenin protein level reduction.
Retinoic acid	Friedman et al. 2016 [115]	RA significantly affects growth, signaling pattern and interactions among PI3K/Bcl2/retinol proteins. β-catenin level depends on the interaction between response to treatment and cell type.
Romina strawberry (anthocyanin fraction)	Giamperi et al. 2019 [116]	Mechanism still unclear. Collagen 1A1, fibronectin, versican, and activin A messenger RNA expression decrease. Pathway connected with signaling in the ECM.
Simvastatin	Borahay et al. 2014 [117] Borahay et al. 2015 [118] Malik et al. 2018 [104]	The decrease of ERK activation and decrease of Ras isoprenylation. The inhibition of the Akt signaling pathway in UF cells. The inhibition of the expression of major ECM proteins – collagen I, collagen III, fibronectin, versican, and brevican.
Quercetin	Greco et al. 2020 [109]	mRNA levels of collagen 1A1 and fibronectin decrease in UF cells. Pathway connected with signaling in the ECM.
Verteporfin	Islam et al. 2019 [119]	The reduction of mRNA levels of versican and MMP-14. Inflammation inhibition, decrease in interleukin 8 (IL-8) levels. TGF-β1 and TGF-β3 mRNA expression levels decreased.