Journal of Tumor Research

Review Article Open Access

Uterine Fibroids: Understanding their Origins to Better Understand their Future Treatments

Carlos Pascual Botía*, Sara Cholvi Camarasa, Francisco Raga Baixauli and Antonio Cano Sanchez

Hospital de Sagunto, Sagunto, Valencia, Spain

Abstract

Despite the high prevalence and the enormous impact the uterine fibroids has on the healthcare system and the economy, there is no effective medical treatment available to eliminate fibroids. This is due in part to the fact that very little is known about their pathophysiology; in order to look for and potentially find treatments capable of eliminating fibroids; we must gain a deep understanding of the mechanisms behind their initiation, stimulation and growth. In this article, we comprehensively review current knowledge of the pathogenesis of uterine fibroids, with the aim of better understanding their origin, as well as discussing the action of already existing medical treatments in order to highlight ways that potential future therapies may be able to target the disease.

A literature search was performed in PubMed to find articles related to the etiopathogenesis of fibroids and their treatments.

Myomas comprise areas of disordered smooth muscle fascicles characterized by an excess of a cellular extracellular matrix (ECM) with a deregulated phenotype. Although the initial event is believed to be smooth muscle cell proliferation, it is thought that a complex signalling system is also necessary. Non-hormonal factors may be responsible for initiating the development of uterine fibroids, although hormonal stimulation is an essential factor for their growth.

Recent studies have confirmed that the different cell types contained in fibroids are all clonally derived from a parental cell with implied multipotent stem cell properties.

Ethnicity also strongly influences the development and clinical severity of fibroids.

Many Growth factors and their respective receptors have been implicated in fibroid growth.

The available evidence continues to reinforce the importance of progesterone in the development of fibroids.

The Ulipristal Acetate mediated volume reduction is due to a series of multifactorial and successive events that lead to a low proliferation rate.

Keywords: Fibroids; Pathogenesis; Genetics; Vitamin D; Extracellular matrix; Progesterone; Ulipristal acetate

Introduction

Uterine fibroids continue to be one of the most common benign uterine tumor types and the most common condition requiring hysterectomy. In the United States, uterine fibroids are the main indication for gynaecological surgery and result in approximately 600,000 hysterectomies and 60,000 myomectomies per year [1]. Nonetheless, the incidence of fibroids in the general population remains unknown, although the literature estimates prevalence between 20 and 40% in women aged over 30 years.

The National Institute of Environmental Health Sciences (NIEHS) uterine fibroid study, performed by ultrasound screening of a randomly selected sample of participants, provided data on the cumulative incidence of fibroids [2]. It documented that black women are more likely than white women to have uterine fibroids, were younger at diagnosis (33 vs. 36 years) and that they also have a proportionally higher number of new diagnoses during screening (59% vs. 43%), resulting in more hysterectomies for fibroids being performed in black women. This study also suggests that more than 80% of black women and about 70% of white women develop uterine fibroids during their forties [1,2]. Interestingly, fibroids also appear to be more common in the United States than in Europe.

In a study by Cardozo et al. [3], published in 2012, data on the monetary cost of fibroids in a population aged etween 25 and 54 years of age in the United States were collected together from previous studies, in order to estimate their annual cost to society. Of this total, surgical management contributed to between $829 million and $4.2 billion, while medical management had cost between $3.27 and $5.1 billion in 2010. The total cost to the health system during this time was $5.89 to $34.37 billion per year, is higher than the cost derived from breast, colon or ovarian cancer and is about one-fifth of the annual cost of diabetes [3]. Moreover, uterine fibroids are clinically apparent in 25% of women and their impact on quality of life is substantial. Attributable symptoms can be classified into three categories: abnormal uterine bleeding, pelvic pain or pressure and fertility disorders.

Despite the high prevalence and the enormous impact this disease has on the healthcare system and the economy, there is no effective medical treatment available to eliminate fibroids. This is due in part to

***Corresponding author:** Carlos Pascual Botía, Hospital de Sagunto, Sagunto, Valencia, Spain, Tel: +34660359405; E-mail: carpasbo@gmail.com

Received September 07, 2017; **Accepted** September 29, 2017; **Published** October 06, 2017

Citation: Botía CP, Camarasa SC, Baixauli FR, Sanchez AC (2017) Uterine Fibroids: Understanding their Origins to Better Understand their Future Treatments. J Tumor Res 3: 130.

the fact that very little is known about their pathophysiology; in order to look for and potentially find treatments capable of eliminating fibroids; we must gain a deep understanding of the mechanisms behind their initiation, stimulation and growth. In this article, we comprehensively review current knowledge of the pathogenesis of uterine fibroids, with the aim of better understanding their origin, as well as discussing the action of already existing medical treatments in order to highlight ways that potential future therapies may be able to target the disease.

Methods

A literature search was performed in PubMed to find reviews and scientific articles related to the etiopathogenesis of uterine fibroids and their most commonly used medical treatments, using the following Boolean search terms: (fibroids[All Fields] AND molecular mechanisms[All Fields]); (fibroids[All Fields] AND pathogenesis[All Fields]); (fibroids[All Fields] AND signalling pathways[All Fields]); (fibroids[All Fields] AND signalling pathways[All Fields]); (fibroids[All Fields] AND vitamin D[All Fields]); (fibroids[All Fields] AND genetics[All Fields]); (fibroids[All Fields] AND progesterone[All Fields]); (fibroids[All Fields] AND united states incidence[All Fields]); (fibroids[All Fields] AND management[All Fields]); (fibroids[All Fields] AND ulipristal acetate[All Fields]); (fibroids[All Fields] AND GnRH analogs [All Fields]).

Only articles or reviews published in English were selected. This search yielded 8545 papers. After eliminating duplicates, two of the team members (FR and CP) screened the titles of these manuscripts to eliminate 5632 more because they were not clearly related to the topics of interest, thus, reducing the number to 1409 potential papers. The same team members then read the abstracts of these papers to remove another 1195 non-eligible articles. Subsequently all of the remaining texts were read and any that were similar to each other but had a lower impact factor or were of lower quality were eliminated. Finally, after careful study of each of the selected papers, we also read other relevant manuscripts cited in them, to eventually include a total of 197 articles in this qualitative synthesis.

Pathogenesis: The Origin and Growth of Myomas

Myomas comprise areas of disordered smooth muscle fascicles characterized by an excess of acellular extracellular matrix (ECM) with a deregulated phenotype [1]. They have abundant eosinophilic cytoplasm and nuclei with a uniform size, shape and chromic staining characteristics; the ECM is usually more abundant in fibroids. Although the initial event is believed to be smooth muscle cell proliferation, it is thought that a complex signalling system is also necessary. Thus, there are at least two components in the development of fibroids: the transformation of normal myocytes into abnormal myocytes and their growth into clinically-apparent tumors [4]. The former is a relatively frequent process because the prevalence of microscopic myomas is high, the latter, i.e. further growth, depends on clonal cellular expansion. There are a variety of subgroups of chromosomes that can be involved and so myomas should be viewed as a common phenotype resulting from different genetic events.

Some studies support the hypothesis that repeated myometrial damage and repair could predispose women to the formation of fibroids [5]. As such, non- hormonal factors may be responsible for initiating the development of uterine fibroids, although hormonal stimulation is an essential factor for their growth [6]. Chronic inflammation may also be a primer for the condition, because increased vascularization and vascular permeability, as well as fibroblast proliferation, are common inflammatory reactions which are also important in the formation of fibroids [7].

The growth rate of myomas in the uterus is not associated with their location or size and progression or regression can occur under the same hormonal conditions. However, whether different etiologies arise depending on the location of the fibroid (e.g. submucosal fibroids harbor fewer chromosomal abnormalities than the intramural or subserosal), remains controversial. In clinical practice, these different types of fibroids can be considered different manifestations of the same disease which differ in symptomatology and in treatment. Thus, submucosal lesions generally respond better to treatment with gonadotropin-releasing hormone (GnRH) analogs and can be excised hysteroscopically.

Many studies assert that the development of fibroids is related to hormonal status: fibroids do not appear in prepubescent women and rarely do so after menopause. In fact, epidemiological studies suggest that there is a lower prevalence in multiparous women and in those who had a late menarche [8]. In addition, research suggests that fibroids develop and/or become more symptomatic in women taking hormone replacement therapy (HRT) at menopause. During pregnancy, despite the presence of high levels of sex steroid hormones, fibroids decrease in size and may become undetectable on postpartum ultrasounds [9]. Another study showed that long-term contraceptive use also decreases risk [4]. Hence, although hormone concentrations are high in both these aforementioned situations, their lower associated risk means that factors other than hormonal concentrations are likely to be important in the development of this disease [10].

Cellular Origin of Fibroids: Candidate Cells for the Development and Growth of Fibroids

Human uterine fibroids are clonal in origin, as confirmed by studying the inactivation of heterozygous-status alleles on the X chromosome [11]. Additionally, recent studies have confirmed that the different cell types contained in fibroids (mainly smooth muscle cells and fibroblasts) are all clonally derived from a parental cell with implied multipotent stem cell properties [12]. The uterus is highly plastic and has a high regenerative capacity both during menstrual cycles and pregnancy, which supports the presence of uterine stem cells. Indeed, one study estimated that approximately 3% of human myometrial cells have stem cell-like properties; these cells were CD34 positive and CD45, CD106, vascular endothelial growth factor receptor 1 (VEGF R1) and antigen-associated factor VII negative. Moreover, 98% of these myometrial cells had a G0 status typical of quiescent stem cells and could grow *in vitro* in hypoxic conditions, another stem cell hallmark [13].

Stem cells from myomas, but not from the myometrium, carry mediator complex subunit 12 (MED12) mutations, meaning that at least one genetic alteration might permit myometrial stem cell transformation into myoma progenitor cells. Furthermore, it has been suggested that uterine hypoxia, aberrant methylation, genetic mutations and abnormal estrogen signaling may play a critical role in the transformation of myometrial stem cells into myomas [14]. Tumors derived from myoma progenitor cells have much higher proliferation rates than those that do not contain these cells. Furthermore, these cells appear to be deficient in estrogen and progesterone receptors (PRs), but have tumorigenic properties when stimulated with these hormones. Fibroid progenitor cells require mature myoma or myometrial cells in order to grow and proliferate [14].

One theory posits that menstrual damage could lead to an abnormal

inflammatory response; for instance, myometrial contractions during menstrual bleeding may induce ischemic damage or damage arising from reperfusion after ischemia in myometrial smooth muscle cells and these cells could be candidates for uterine fibroid progenitor cells. In support of this hypothesis, the presence of cells positive for apoptotic markers, p53 and p21 is limited to the follicular phase of the menstrual cycle, whereas proliferative Ki-67 positive cells are mainly observed in the luteal phase. These studies suggest that most damaged cells (apoptotic cells or cells in cell-cycle arrest) are eliminated during the follicular phase but that some of them may survive, acquire mechanisms which protect them against oxidative stress and apoptosis and become uterine fibroid precursor or progenitor cells [15,16].

Role of Genetics in the Development of Fibroids

Despite their benign nature, approximately 40-50% of fibroids contain chromosomal, karyotypic and cytogenetic abnormalities that are not random or tumor specific [16-20]. On the other hand, 50% have a normal karyotype and so other pathogenic mechanisms must exist [18,20]. Recent studies have sequenced the complete genome and gene expression profile of human uterine fibroids, to identify many different genes and chromosomes associated with their presence [21]. Importantly, cytogenetic analysis indicates that individual fibroids are likely clonally derived from an original tumor [17]. Moreover, the demonstration of mosaicisms in clonal-origin myomas also supports the hypothesis that chromosomal instability plays a secondary role during fibroid growth. Myomas with a normal karyotype are usually smaller than those with chromosomal abnormalities [22] and the absence of cell mosaicism often produces larger myomas than those with mosaicisms [18].

Clinical and observational studies have shown that genetic or chromosomal alterations play a significant role in the development of uterine fibroids and family and twin studies support this fact: first-degree relatives of women with fibroids have a higher risk of developing fibroids [23] and there is more concordance in the development of myomas in monozygotic twins than dizygotic twins [24]. Importantly, the presence of fibroids is also more common in several genetic syndromes in which the development of smooth muscle tumors is characteristic, such as in Alport Syndrome [25,26].

Epigenetic Factors

Epigenetic factors are heritable changes in the expression of genes that are not encoded in the DNA sequence [16].

DNA methylation

The results obtained so far are disparate and indicate a possible functional role for DNA promoter methylation in silencing genes involved in the pathogenesis of uterine fibroids [16].

microRNA

microRNAs are stable, non-coding, single-strand RNA pairs that are approximately 22 nucleotides long which regulate gene expression through gene silencing. This post-transcriptional regulation seems to play a significant role in multiple female reproductive system pathologies. For example the expression of several microRNAs (including let7, miR-21, miR-93, MiR-106b and miR-200) and their target genes are altered in uterine fibroids and adjacent myometrium [16,27-29]. It is currently thought that the initial event in the de novo formation of uterine myometrial fibroids is simple genetic alterations which cause the formation of complexes of multiple chromosomal breaks and random rearrangements [30]. However, whether the underlying mechanism is hereditary, acquired or a complex combination of congenital alterations and acquired cellular mutations remains unknown [31].

MicroRNA expression appears to strongly depend on epidemiological features such as myoma size and ethnicity: e.g. fibroids from African-American women have a distinct microRNA expression profile compared to Caucasian women [32]. It is possible that differential microRNA expression could be used in future applications as diagnostic biomarkers for differentially diagnosing myomas and leiomyosarcomas [33]. The identification of microRNA biomarkers could also help to guide the development of effective long-term treatments to prevent the progression of the disease or to predict individual responses to different treatments [33].

Finally, the expression of microRNAs and RNAs shows an inverse association pattern, thus implicating multiple pathways, including cell proliferation control, mitogen-activated protein kinases (MAPKs) and transforming growth factor (TGF)-B and Wnts, among others.

Cytogenetic aberrations in fibroids

The genes most frequently involved in the genesis of fibroids are (MED12) and high mobility group AT-hook 2 (HMGA2).

Mediator complex subunit 12 and its interacting proteins

MED12 is a component of the regulatory complex that has a role in regulating RNA polymerase II [32]. MED12 is located on the X chromosome and up to 70% of the fibroids found in two separate cohorts of Finnish and South African women contain a MED12 mutation. It also appears that mutations in exon 2 of MED12 are particularly prevalent in fibroids, although this mutation is also found in 11% of muscle tumors with an uncertain malignancy status and in 20% of uterine leiomyosarcomas [1,16]. MED12 plays a role both in activation and transcriptional repression of many developmental mechanisms [16].

MED12 mutation in fibroids is mostly associated with normal tumor karyotype, however, it remains to be seen whether this mutation leads to the development or growth of myomas; it is known to be associated with the presence of smaller tumors, suggesting that MED12 dysfunction leads to a generally inhibitory effect on gene expression [34]. This was also confirmed *in vitro*, where cultured myomatous cells harboring a MED12 mutation tended to stop proliferating after a few cycles. MED12 inactivation results in TGF-B stimulation, correlating with the 3-fold higher expression of TGF-B found in leiomyomas compared to the myometrium [30]. TGF-B affects genes which produce collagen proteins and therefore also plays a role in ECM formation [31].

Translocation t(12;14) (q14-15;q23-24), HMGA2 and RAD5IB

About 20% of myomas with abnormal karyotypes have translocations between chromosomes 12 and 14, t(12;14) (q14-15;q23-24) [35]; HMGA2 on chromosome 12 is a target gene and RAD5IB on chromosome 14 is its most common translocation partner [36,37]. HMGs are proteins that act as regulatory transcriptional factors and are involved in several important cellular processes such as growth, proliferation, differentiation and cell death. High-mobility group AT-hook 2 (HMGA2) is frequently expressed in uterine fibroids with 12q15 chromosomal rearrangements; it is one of the target genes of the let-7 family of microRNAs and is often suppressed *in vitro* by let-7 [1,16,38].

Cumulative evidence indicates that HMGA2 plays an essential role in stem cell renewal and functions as a proto-oncogene [19,39]. HMGA2 is highly expressed during embryonic development, although it is also found in a wide variety of benign and malignant tumors. Moreover, this gene is overexpressed in myomas containing the t(12;14) (q14-15;q23-24) mutation [40] whose presence is associated with increased fibroid size [18]. HMGA2 protein overexpression and let-7 microRNA under expression was observed in fibroids larger than 10 cm compared to those less than 3 cm. Moreover, the introduction of let-7 microRNA in cultures of cells from these large myomas (with relative HMGA2 overexpression) resulted in a reduction of HMGA2 protein. However, it is hard to imagine that *in vivo* regulation of HMGA2 by let-7 has a specific functional role in fibroids or is the only factor contributing to tumor genesis and growth [11,31].

Despite advances, it is unclear why the t(12;14)(q14-15;q23-24) translocation is the most frequent cytogenetic aberration found in fibroids or why it is associated with increased fibroid size. One hypothesis is that this translocation does not represent a mere side effect, but that rather, it is an important causal factor in the development of fibroids, for example, by its induction of b-FGF mRNA expression [41]. Similarly, the RAD5I recombinase is essential for double-stranded DNA repair and homologous recombination during meiosis. Hence, alteration of RAD5I is more likely to be a cause rather than an effect of chromosomal alterations and the fusion protein resulting from the translocation of this gene (RAD5I-HMGA2) does not represent the pathophysiological mechanism causing the development of fibroids [37].

Ethnic/racial differences in fibroid biology

Ethnicity also strongly influences the development and clinical severity of fibroids. Multiple studies have shown that African-American women are more likely to develop fibroids and that these develop at a younger age, in greater number, are larger and more symptomatic (more severe) and are more likely to be treated by hysterectomy in this racial group compared to Caucasian women [2,42-46]. This may be due to a combination of specific genetic factors and environmental factors that are not independent risk factors, but its etiology still remains to be fully explored (Figure 1 and Table 1).

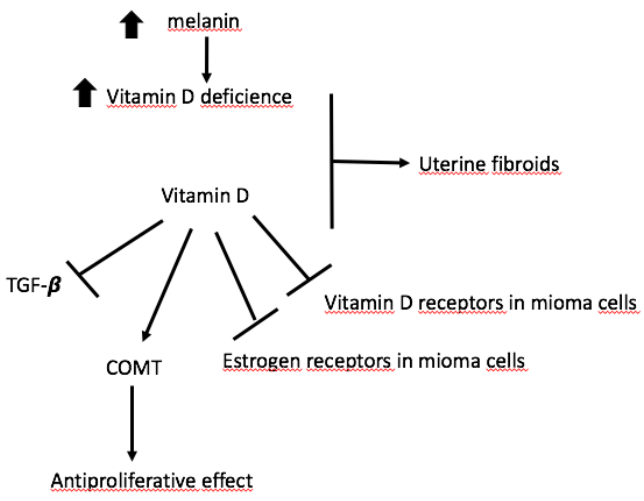

Figure 1: Ethnic/racial differences in fibroid biology.

Racial differences
African American Women and Fibroids
• More likely to develop fibroids
• At younger age
• In greater number
• Larger
• More symptomatic (more severe)
• More chances of hysterectomy
• Vitamin D deficiency is 10 times higher

Marshall LM et at., 1997; Nesby-O'Dell et al 2002; Baird et al., 2003; Moorman et al., 2013; Baird et al.,2013; Marsh et al., 2013; Mitro et al., 2015.

Table 1: Summary racial differences.

Differences at the genetic level: Catechol-O-methyltransferase expression

Increased aromatase activity and cytoplasmic carbonic anhydrase (CAIII) gene expression have been observed in rapidly growing fibroids in African-American women. Hence, the fibroids common in this population are thought to comprise cells adapted to survive in acidic and hypoxic environments and which are resistant to apoptosis pathways [6,47,48]. In addition, other studies have reported a role for ethnicity-associated polymorphisms in the alpha estrogen receptor (ESR1) gene, as well as for significantly higher increased catechol-O-methyltransferase (COMT) Val/Val genotype activity in myomatous tissue compared to surrounding myometrial tissue, in African-American women, the latter being associated with a 2.5-fold increased risk of developing fibroids [49]. In particular, one COMT allele (Vall58) produces a highly active protein and is commonly found in African-American women. The COMT enzyme is essential for estrogenic metabolism and thus, may cause or be involved in the formation of fibroids. Indeed, a recent Meta-analysis confirmed the association between specific COMT polymorphisms and uterine fibroids [50].

Vitamin D

It is still not known if vitamin D is a risk factor which contributes to the racial differences in fibroid presentations. The prevalence of vitamin D deficiency is 10 times higher in African-American women than in Caucasian women [51] and several recent epidemiological studies have suggested an inverse relationship exists between serum 25-hydroxycholecalciferol (vitamin D) levels and the prevalence of uterine fibroids, both in black and white women [43,52,53]. This relationship is also found among infertile women with vitamin D deficiency and among those taking some types of treatments for symptomatic fibroids [43,52,53]. Dark-skinned women are more likely to have vitamin D deficiency because of the higher concentration of melanin contained in their skin — the prevalence is 80% in non-Hispanic black women vs. 20% in white women [54]. Therefore, several studies have shown a significant inverse relationship between serum vitamin D levels and fibroid severity in African-American women: the lower the vitamin D level, the greater the fibroid severity [43,52,55].

Vitamin D treatment has been shown to inhibit myometrial cell growth *in vivo* and to decrease the size of myomas in the Eker rat model. The anti-proliferative effect of vitamin D in myomas *in vivo* is caused by modulation of COMT expression and protein activity [56-59]. At the cellular level, *in vitro* treatment of fibroids with biologically-active vitamin D (1,25-dihydroxyvitamin D3) inhibits their cell growth. Correspondingly, myoma cells express lower levels of vitamin D receptors (60% of cells) with respect to the adjacent myometrium [58-61]. This may be caused by the loss of vitamin D function, caused by its

deficiency and lower vitamin D receptor expression and might be an important risk factor for fibroid presentation [55].

However, in contrast to the above, the relationship between vitamin D and uterine fibroids was not observed in an important study based on the NHANES (National Health and Nutrition Examination Survey) population. Population-adjusted analyzes in premenopausal population, which included women of all races and ethnicities, failed to find a consistent association between vitamin D and the presence of fibroids. Thus, it differed to the previous studies because fibroids were diagnosed solely by ultrasound and so both symptomatic and asymptomatic women were included. However, those with very small fibroids were excluded because they were not identified by the ultrasound. In addition, this study cohort was less varied and more homogeneous in terms of access to healthcare resources [46].

Vitamin D3 inhibits growth and induces apoptosis in cultures of human myomatous cells by downregulating proliferating cell nuclear antigen (PCNA), CDk1 and BCL-2 and suppressing COMT expression and activity [58]. In addition, vitamin D suppresses the role TGF-B plays in the process of fibrosis formation in human myomatous cells [61]. More than 60% of myomatous tissue analyzed expresses low levels of vitamin D receptors compared to adjacent normal myometrial tissue [61]. In addition, *in vivo* treatment of cells with vitamin D3 induces the expression of vitamin D receptors in a concentration-dependent manner. Vitamin D reduces the abnormal expression of smooth muscle fibers in human myomatous cells, as well as reducing the expression of sex steroid receptors in a concentration-dependent manner in myoma cells [62]. Taken together, the findings of these *in vivo* and *in vitro* studies suggest that vitamin D should be considered an effective, safe, non-surgical and long-term therapeutic option for medically treating fibroids [55].

Growth Factors

Growth factors (GFs) are proteins or peptides, which are produced locally by smooth muscle cells and fibroblasts and interact in various cellular events such as proliferation, extracellular matrix synthesis and angiogenesis, all of which are important for fibroid growth [16]. Many GFs and their respective receptors have been implicated in fibroid growth, including heparin-binding epidermal GF-like GF (EGF), platelet-derived GF (PDGF), insulin-like GF (IGF), transforming GFs a and b (TGF-a and TGF-b), vascular endothelial GF (VEGF), fibroblast GF (FGF) and basic FGF [63].

Growth factor receptors

Receptor tyrosine kinases: Receptor tyrosine kinases (RTKs) are surface GF receptors. They are play important roles in regulating processes like proliferation, differentiation and metabolism. Several types of RTKs are overexpressed in fibroids and most of them are over-phosphorylated compared to normal myometrium. Moreover, estrogen expression causes an increase in expression of GFs and their related RTKs in fibroids which thus, represent intermediate effectors for the effects of sex steroids on fibroids [64,65].

Growth factor signalling pathways

The Ras/Raf/MEK/ERK pathway: This signaling pathway regulates several critical processes, including cell proliferation and survival [66]. There is a complex and bidirectional interaction between steroids and the Ras/Raf/MEK/ERK pathway: estrogens can induce ERK pathway activation and GFs are able to modulate the steroid hormone response through the effects of ERK on the transcriptional activity of steroid receptors. Rapid 17B-estradiol signaling is associated with the activation of ERK in myoma cells, while in myometrial cells it diminishes phosphorylated ERK [67,68].

PI3K/Akt/mTOR pathway: There is recent evidence that aberrant PI3K/Akt/mTOR signaling in fibroids plays a role in fibroid pathophysiology. Individual role of growth factors in the pathophysiology of fibroids epidermal growth factor and platelet-derived growth factor. Epidermal growth factor (EGF) enhances DNA synthesis and polyploidization in myoma cells through transient activation of the EGFR-MAPK (mitogen activated protein kinase) pathway. Platelet-derived growth factor (PDGF) stimulates DNA and protein synthesis, increases the expression of collagen a1 and PCNA, activates the MAPK pathway and also modulates cell proliferation in the myometrium and in myoma cells. This suggests that it contributes not only to proliferation but also to the excessive deposition of ECM in fibroids [69-72].

Transforming growth factor B: There is evidence demonstrating the role of alterations in transforming growth factor B (TGF-B) signaling in the growth and development of uterine fibroids, which show a more than 5-fold increase than in myometrial TGF- B expression. It has a bimodal effect on cell proliferation and induces the expression of genes related to ECM production (and reduces the expression of genes whose proteins degrade ECM). TGF-B also activates the MAPK pathway and in small amounts, increases cell proliferation by stimulating autocrine secretion of PDGF [71].

Myomas have altered TGF signaling pathways and are refractory to the antiproliferative effects of TGF-B1 and B3 that are seen in normal myometrium. The presence of TGF-B neutralizing antibodies decreases collagen type I and III levels in myomas and myometrial cells and TGF-B3 induces the secretion of fibronectin by myomatous cells and stimulates cell proliferation both in myometrial and myomatous cells [73,74]. Drugs such as GnRH agonists (GnRHas) and asoprisnil interact with these GFs. For example, asoprisnil inhibits proliferation and induces myoma cell apoptosis and decreases the expression of EGF, IGF1 and TGF-b mRNA and proteins in fibroids but not in the myometrium. Therefore, it is believed that the therapeutic effect of this drug is mediated via downregulation of the expression of these GF in fibroids [75,76].

Insulin-like growth factor I: IGF1 is mitogenic and increases the proliferation of myoma cells by activating the MAPK pathway. It plays a crucial role in the formation of fibroids by stimulating Bcl-2 protein expression in myomatous cells. Both IGF2 and IGF1 transcript mRNA levels are increased in fibroids but only IGF2 protein levels concurrently increase in these cells. Moreover, IGF1 and p-AKT overexpression correlates with myoma size; IGF1 signaling appears to be regulated by estrogens [59,77,78].

Vascular endothelial growth factor: VEGF-A is significantly overexpressed in myomatous tissue compared to adjacent myometrial cells and this GF is required for myoma growth in *in vivo* xenografts. It may represent a therapeutic target for fibroid treatment and its expression decreases upon treatment with selective PR modulators [79-81].

Cytokines: Cytokines are low molecular-weight proteins produced by immune system cells [82]. Interleukins 11 and 13 are the most relevant in the context of fibroid pathogenesis: they are overexpressed in myomas compared to the myometrium and serve as key regulators in subepitelial fibrosis, particularly by interacting with TGF-B [83,84]. There are significant differences in the interleukin IL-Iâ and IL-12 â-receptor polymorphisms found in women with a diagnosis of pathological myomas versus controls [85,86].

Extracellular matrix components: Myomas are characterized by qualitative and quantitative abnormalities in ECM components (which account for most of the tumor volume and symptomology), including collagen, fibronectin and proteoglycans [16]. Not only is their production excessive, it is also unordered and has an altered composition compared to that of healthy tissues. Myomas contain 50% more ECM than the myometrium and can serve as a reservoir for the GFs, cytokines, inflammatory and angiogenic mediators and proteases produced by these altered cells [87]. Collagens are the major component of the ECM [88]; fibroids have an abnormal fibroblast structure and orientation, overexpress type I and III collagen mRNA and increased levels of type I and V collagen proteins compared to the adjacent myometrium [32]. Furthermore, fibroid growth can be partially mediated via autocrine and paracrine mechanisms. Tumor-derived fibroblasts and myoma cells promote the synthesis of GFs and activate signaling pathways which are important in cell proliferation and the production of ECM components. In addition, ECM directly stimulates intercellular signaling: the abnormal composition, structure, fluid content and stiffness of the tumor, causes an increase in tissue tension (mechanical stress). In turn, this increase in voltage and shock induces mechanical signals that are transmitted from the collagen and other ECM fibers to intracellular components via transmembrane receptors. This complex signaling pathway induces alterations in cell and cytoskeletal shape, changes ECM stiffness and may contribute to increased cell growth [32,59,89].

Dermatopontin and proteoglycans are found in fibroids and keloids and both share epidemiological characteristics: the first is seen in lesser amounts than in the myometrium while the second is more in the normal myometrium [89,90]. Fibronectin is a glycoprotein that binds collagen to integrins, but there is disagreement on how much is expressed in the myometrium. However, TGF-B3 has been shown to induce the expression of fibronectin in myomatous cells and directly stimulates cell proliferation of myoma and myometrial cells in culture [91].

Other signaling pathways

Wnt/â-catenin: Myomas with MED12 mutations overexpress Wnt (wingless-type) family proteins including Wnt14. On the one hand, wnt/â-catenin signaling mediates an interaction between myomatous stem cells (representing 1% of tumor cells) and mature myomatous cells that promote tumor growth. On the other hand, estrogens and progesterone induce Wnt 11 and 16 expressions in mature myomatous cells which, through paracrine effects, lead to nuclear translocation of â-catenin in stem cells and subsequent transcription of their target genes, including AXIN2 and thus results in stem cell proliferation. Therefore, estrogens and progesterone induce proliferation in fibroids which is modulated, in part, through Wnt expression in mature cells and their paracrine effect on â-catenin signaling in stem cells [92-94].

Retinoic acid: Retinoic acid (RA), the active metabolite of vitamin A (retinol), is involved in various biological functions, especially those related to growth and development. Myoma cells are retinoid responders because they can develop receptors involved in RA signaling and all-trans retinoic acids inhibit their proliferation. As compared to the myometrium, myomatous cells express several genes coding for enzymes and RA signaling pathway receptors. Moreover, treatment with LGD1069, a retinoid X receptor-selective ligand, reduces the size of myomas in the Eker rat animal model [95,96].

Peroxisome proliferator-activated receptors: Peroxisome proliferator-activated receptor upsilon (PPARϒ) signaling inhibits myoma growth by modulating estrogen signaling, thus, PPAR signaling may modulate myoma growth and could represent a potential therapeutic target [96].

Hormones: Progesterone and estrogen

To date, the true functional role of estrogen and progesterone in the biology of fibroids is still not fully understood (Figure 2).

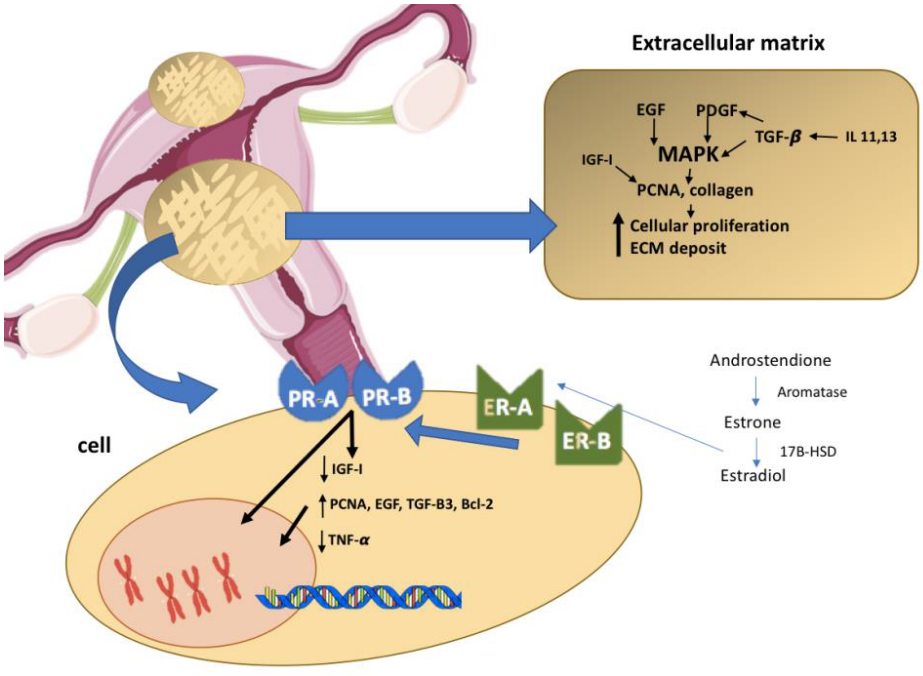

Figure 2: Functional role of estrogen and progesterone in the biology of fibroids.

Estrogens: Estrogens have long been implicated in fibroid pathophysiology because of their ubiquitous presence in women during reproductive-life stages, their absence before puberty and substantial reduction after menopause. In addition, fibroids treated with GnRHas (which are known to suppress estrogen production) decrease in size [6,59,97,98]. Several groups found increased levels of estrogen receptor alpha and beta (ERa and ERb) mRNA and protein in myomas compared to normal myometrium [47,99,100], although recent studies were unable to replicate these findings [16,101].

Estrogens can alter the expression of many genes, including c-fos, c-jun and IGF1. Their cellular effects are mediated through two pathways: modulation of transcriptional activity (mainly through nuclear receptors) and rapid signal translation (usually membrane mediated), although both receptors can function in both ways [102]. Some authors suggest that estrogens can maintain PR levels and thus, that progesterone promotes fibroid growth through its receptor [103]. Importantly, estrogens can also decrease tumor suppressor protein (p53) expression in myomatous cell cultures, which may play a role in stimulating myoma growth [104]. Similarly, estrogens might also promote myoma cell proliferation by regulating GFs (e.g. they stimulate PDGF expression) and signaling pathways, for example, by inhibiting activin and myostatin expression [16,105,106].

Fibroids overexpress estrogen and GPR30 receptors [107] which are more frequently phosphorylated in myomas than in the myometrium. Therefore, these phosphorylated receptors (perhaps regulated through rapid but aberrant p44/42 MAPK signaling) might have an important role in leiomyoma development [108]. Human smooth muscle and uterine fibroid cell proliferation is dose-dependently stimulated by 17â-estradiol, which may be sensitive to inhibition by rapamycin or LY294002 (a phosphatidylinositol-3 kinase [PI3K] inhibitor); this means that estrogens exert their pro-mitogenic effect via transcriptional mechanisms [109,110] which could perhaps be exploited therapeutically.

Nevertheless, a recent meta-analysis concludes that there is currently insufficient evidence to show whether selective estrogen receptor modulators can truly reduce tumor size or have any clinical benefits [1,111]. Even so, several therapeutic options aim to reduce myoma size by decreasing estrogen levels (e.g. GnRHas, aromatase inhibitors and selective estrogen receptor modulators). However, these protocols are of limited use because of the unwanted side effects produced by the resulting hypoestrogenism. Another potential future therapeutic option is the use of adenoviruses to confer a predominantly negative state onto estrogen receptor genes which may inhibit myoma growth [59].

Aromatases, estrogens and estrogen receptors: Fibroids contain significantly higher levels of 17B-hydroxysteroid dehydrogenase (17B-HSD) type I and aromatase enzyme than the adjacent myometrium. *In vitro*, the addition of androstenedione to myomatous cell cultures allows estrone production which is then converted to estradiol via 17B-HSD. When androstenedione is added to these cultures, cell proliferation increases in a similar way to the addition of estradiol, but addition of aromatase inhibitors decreases this proliferation. Thus, it is likely that aromatase is required for fibroids to produce sufficient estrogens to be able to autonomously sustain their own growth [112,113].

Progesterone: The weight of available evidence continues to reinforce the importance of progesterone in the development of fibroids [59]. Although estrogens were originally implicated as the primary stimulus in fibroid growth, clinical studies and mouse models have shown that progesterone is necessary for estrogen- related fibroid growth, suggesting that estrogens are required, but not sufficient, to stimulate fibroid proliferation [114,115]. In addition to the alterations both of these steroids induce, they also interact with each other: estradiol induces the expression of PRs in myomatous cells [59,115].

There is also evidence that progesterone interacts with GFs, which may influence fibroid growth: surprisingly, progesterone inhibits IGF1 expression in myomatous cells and increases expression of cell proliferation regulatory genes, including PCNA, EGF and TGF-B3 [116-118]. It also stimulates apoptosis regulator B-cell lymphoma (Bcl-2) protein expression while decreasing tumor necrosis factor alpha (TNF-a) expression. Furthermore, progesterone can rapidly activate the AKT oncogene pathway in fibroid cells, inducing cell proliferation [16,118-121]. In addition, proliferation markers such as Ki67 and PCNA are increased in luteal or secretory-phase in myomas, which express higher levels of progesterone [122-124]. Further to this, clinical studies have shown that the quantitative proliferation rates of fibroid cells is significantly higher in postmenopausal women receiving combined estrogen and progesterone hormone replacement therapy (HRT) rather than estrogen alone or no HRT [115,124]. Similarly, fibroid size tends to increase during the first 10 weeks of pregnancy when progesterone levels are elevated [125,126], but does not significantly change size between second and third trimester when they stabilize and reduce.

Taken together, current evidence for the role of progesterone in fibroid biology is contradictory. However, these data, coupled with the fact that many fibroids are clonal and can have different growth patterns in the same uterus, suggest that both local and cellular mechanisms play key roles in the development and growth of fibroids [9,127].

Progesterone receptors: Similar to estrogen, progesterone is generally considered to render its effects via both its progesterone receptor (PGR) gene isoforms, PR-A and PR-B, which belong to the family of ligand-activated transcription factors and share structural and functional elements with other steroid hormone receptors. Higher levels of PR mRNA and proteins are expressed in myomas compared to normal myometrium [128-131], however, PR-B mRNA is often expressed in the surface cells of fibroids, suggesting fibroid growth has an active progestacional-proliferation phenotype [132].

Both receptors affect cell function by altering gene expression through one of two predominant pathways: modulation of nuclear transcription and activation of rapid signaling. In the former, direct genomic route, PRs act as ligand-activated transcription factors that directly interact with specific DNA promoter and transcription regulators to modulate gene expression. In the latter, indirect extranuclear route, PR interacts with cytoplasmic Src tyrosine kinases to activate MAPKs, which then intervene in gene expression [133].

In response to progesterone, PR-B is a strong transactivator, whereas PR-A is less active and often inhibits PR-B, especially when its levels exceed those of PR-B and thus, the PR-A:PR-B ratio is more than 1 [134,135]. These two receptors have opposing transcriptional activity and as such, net progesterone responsiveness is inversely related to the PR-A:PR-B ratio. Hence, this mechanism allows progesterone response to be controlled by modulating the expression levels of their isoforms on target cells [136]. Expression of PGR (encoding PR-A and PR-B) in uterine cells is stimulated by estrogen via the ER-a pathway and consequently, progesterone response is dependent on the presence of estrogens. However, ER expression on uterine cells is inhibited by the PR pathway. This functional feedback between the estrogen and

progesterone hormone systems is crucial for normal uterine function and to compensate for the opposing effects of the P/PR and E/ER systems [136].

Role of Progesterone in the Growth and Maintenance of Uterine Fibroids *In Vitro*

In their *in vitro* xenograft study, Ishikawa and colleagues found that the volume of myomas treated with estradiol and progesterone (E2+P4) was significantly higher than those treated with estradiol (E2), but that this effect was completely blocked by co-administering the PR antagonist, RU486. They therefore concluded that P4 induces fibroid growth through the PR pathway. In addition, only the E2+P4 combination produced significant differences in tumor size (even when treated at supraphysiological E2 doses) and the Ki67 index was also significantly higher in this group, confirming that both E2 and P4 are required to stimulate myomatous growth via myoma cell proliferation *in vitro*. However, cell density was also significantly lower in this group, thus suggesting that an increase in ECM volume or cell size also contributes to tumor enlargement. E2 receptors were expressed in all the treatment combination groups, whereas PRs were expressed only in E2-dependent xenografts. Moreover, PR expression was higher in the E2+P4 ± RU486 groups, indicating that E2 is essential for P4 function in myoma cells *in vivo*. When E2 and/or P4 were withdrawn, both xenograft volume and Ki67 expression significantly reduced; apoptosis did not increase, rather, cell size significantly reduced and cell density increased, thus representing a potential mechanism for fibroid size reduction in these tumors. Therefore, these studies show that estrogens and progesterone increase the growth of myomas by increasing the number of cells and the volume of ECM. It appears that the peak of increase in cell proliferation occurs at the beginning of fibroid growth, whereas the increase in ECM appears constant [115].

Current Medical Treatments

Approximately 30% of women with fibroids require treatment for their symptoms, which can include heavy menstrual bleeding and abdominal pain [137]. Several drugs aimed at alleviating fibroid-derived symptoms or with curative intention are currently under investigation. Here we review the state of the art for two of the most relevant treatments in this field: GnRHas and ulipristal acetate (UA) as well as the pathways involved in their action, relating this information to the previously described fibroid physiopathology to gain insight into their function and outlooks for their possible future use.

Gonadotropin-releasing hormone agonists

Repeated or continuous (non-pulsatile) GnRHa administration, finishing with supra-physiological doses, inhibits the pituitary-gonadal axis [138] which restores hemoglobin levels and decreases fibroid size [97,139] in direct proportion to the percentage of cells with estrogen receptors [140]. GnRHa treatment also decreases arterial size and blood flow variables and causes fibroid hyalinization [141]. However, this treatment has the negative effect of inducing hypoestrogenism and temporary menopause with amenorrhea and so cannot be used long-term; after GnRHa discontinuation leiomyomas again increase in size [138,142]. A recent study suggests that adjuvant therapy (tibolone, raloxifene, estriol and ipriflavone) may help reduce bone loss and that medroxyprogesterone acetate (MPA) and tibolone may attenuate vasomotor symptoms [143].

The use of these drugs prior to surgery remains controversial, but may be beneficial, especially so in submucosal myomas, because they can resolve anemia, decrease fibroid size and reduce endometrial thickness and vascularization, consequently, improving the visual field for hysteroscopy and reducing fluid absorption during surgery [97,137,139].

However, a recent meta-analysis analyzing GnRHa use prior to myomectomy by hysteroscopy, Kamath et al. [144] concluded that the evidence presently available is insufficient to recommend its use and those further clinical trials are still necessary. Another study that compared the potential benefits of administering the aforementioned preparative treatments prior to myomectomy by hysteroscopy, Bizzarri et al. [145] concluded that triptorelin, letrozole and UA all reduced myoma volume, but that UA did so the least efficiently. Nonetheless, myoma resection was complete in all the groups that received preoperative treatment and the perceived surgical difficulty was lower for the letrozole and triptorelin groups (less time was spent in surgery and the infused fluid volume was lower).

Selective progesterone receptor modulators

As the role of progesterone in the origin and growth of fibroids is becoming more established, the search for medical therapies to eliminate fibroids based on this pathway is intensifying. Hence, the antiprogestin UA, belonging to the selective PR modulator (SPRM) family, is now the drug most commonly studied in clinical settings for this disease. Clinical trials have demonstrated that modulation of PRs with drugs such as mifepristone and UA decrease the size of fibroids and alleviate the clinical symptoms they cause [146,147]. In comparison with GnRHas, UA produces longer-term volume reductions after treatment discontinuation, although agonists generally achieve greater overall volume reductions [16].

The mechanisms of action of these drugs are still being investigated. Although the expression of the tumor suppressor gene Krüppel-like transcription factor 11 (KLF11) is significantly lower in myomas than in the adjacent myometrium and so its dysregulation may be a key factor in fibroid pathophysiology. In cell cultures SPRM treatment decreases cell proliferation by reducing the expression of GFs and their receptors, including IGF1, EGF, TGFB3, VEGFA and VEGFB. These treatments also induce cell apoptosis by activating several cell death pathways. Similarly, asoprisnil also decreases PCNA expression while increasing the percentage of terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate (dUTP) nick end labeling (TUNEL) positive cells, decreases anti-apoptotic Bcl-2 protein expression and increases the presence of active caspase-3. All of these effects are related to induction of apoptotic signals and inhibition of proliferative signaling pathways and occur in fibroids but not in the myometrium [148].

Several pharmacokinetic studies have demonstrated that UA is safe in multiple doses [137,149,150] and large clinical trials have shown promising results for this drug in terms of efficacy and safety [149,150]. A 3-month cycle of UA was able to control uterine bleeding and correct anemia in more than 90% of patients within a mean time of 5-7 days (as compared to 21 days for GnRHa) and this effect was sustained for up to 6 months in women who did not undergo surgery this initial 3-month treatment. In contrast, the fibroids returned to their pre-treatment dimensions within 6 months of treatment discontinuation in women treated with GnRHas [137,149,150]. Interestingly, PR modulator associated endometrial changes (PAECs) — a benign effect that disappears within 2 months of ending the treatment — appeared in almost 70% of women taking UA.

Mechanisms of ulipristal acetate action

Progesterone has a dual effect on the growth of fibroids: on the

one hand it promotes their growth by stimulating EGF and Bcl-2 and decreasing the TNF-a expression, but on the other, it inhibits their growth by decreasing IGF1 expression [128,131]. One study examined how UA could cause this decrease in fibroid size by comparing UA-treated myomas to untreated controls and uncovered a widely varying response to treatment because fibroids in the same uterus responded differently to UA. The median Ki-67 index in UA-treated myomas tended to be lower than in untreated fibroids, but this difference did not reach statistical significance [151]. Of note, cyclin-dependent kinase 2 was also involved in cell growth inhibition in UA-treated myomas [152].

In cell cultures, UA stimulates caspase-mediated apoptosis. An increase in dead cells upon UA treatment has also been reported, but so far it has been impossible to demonstrate this through the caspase-mediated TUNEL technique [151]. Only a few TUNEL positive cells were detected in myomas treated long-term with UA, thus reinforcing the hypothesis that UA brings about a limited period of apoptosis-mediated cell death by cell selection, independently of caspase action. The former case would result in selection of cells which are more susceptible to death, meaning that the surviving cells may be UA resistant, thus, justifying the lower percentage of TUNEL-positive cells observed. Together, this evidence indicates that cell death does occur during UA treatment and that this may contribute to the fibroid volume reduction seen during the first weeks of UA treatment [151].

The same authors also showed an overall reduction in the ECM volume fraction in fibroids after prolonged UA treatment compared to untreated fibroids. This is consistent with the increase in cell density observed when the ECM was less abundant and the fact that collagen content tends to decrease in myomas treated long-term with UA. These results suggest that resorption of myoma ECM requires repeated and long-term UA administration [151]. In cell cultures, UA stimulates expression of matrix metalloproteinase 2 (MMP-2), which is directly regulated by progesterone; its expression increases in UA-treated fibroids in a time-dependent manner, suggesting that UA somehow regulates MMP2 expression [151]. This also identifies MMP-2 as an important mediator in the ECM reabsorption required reducing the volume of fibroids.

Hence, Courtoy and colleagues showed that the UA-mediated volume reduction is due to a series of multifactorial and successive events that lead to a low proliferation rate, a transient stimulation of cell death that is not dependent on caspase 3 and a dramatic reduction in the ECM (especially notable after long-term treatment), which could be partially explained by the increased MMP2 expression they detected [151].

Conclusion

Despite the high prevalence and the enormous impact the uterine fibroids has on the healthcare system and the economy, there is no effective medical treatment available to eliminate fibroids. This is due in part to the fact that very little is known about their pathophysiology; in order to look for and potentially find treatments capable of eliminating fibroids; we must gain a deep understanding of the mechanisms behind their initiation, stimulation and growth.

Myomas comprise areas of disordered smooth muscle fascicles characterized by an excess of acellular extracellular matrix (ECM) with a dysregulated phenotype. Although the initial event is believed to be smooth muscle cell proliferation, it is thought that a complex signalling system is also necessary. Non-hormonal factors may be responsible for initiating the development of uterine fibroids, although hormonal stimulation is an essential factor for their growth.

Recent studies have confirmed that the different cell types contained in fibroids are all clonally derived from a parental cell with implied multipotent stem cell properties. 40-50% of fibroids contain chromosomal, karyotypic and cytogenetic abnormalities that are not random or tumor specific. The genes most frequently involved in the genesis of fibroids are (MED12) and high mobility group AT-hook 2 (HMGA2).

Ethnicity also strongly influences the development and clinical severity of fibroids. Several studies have shown a significant inverse relationship between serum vitamin D levels and fibroid severity in African-American women.

Many Growth factors and their respective receptors have been implicated in fibroid growth. Myomas are characterized by abnormalities in ECM components and can serve as a reservoir for mediators produced by these altered cells (fibroid growth can be partially mediated via autocrine and paracrine mechanisms).

The available evidence continues to reinforce the importance of progesterone in the development of fibroids (suggesting that estrogens are required, but not sufficient, to stimulate fibroid proliferation).

In comparison with GnRHas, UA produces longer-term volume reductions after treatment discontinuation, although agonists generally achieve greater overall volume reductions. The Ulipristal Acetate mediated volume reduction is due to a series of multifactorial and successive events that lead to a low proliferation rate, a transient stimulation of cell death that is not dependent on caspase 3 and a dramatic reduction in the ECM (especially notable after long-term treatment), which could be partially explained by the increased MMP2 expression.

Approximately 30% of women with fibroids require treatment for their symptoms. Several drugs aimed at alleviating fibroid-derived symptoms or with curative intention are currently under investigation. Here we have reviewed the state of the art for the most relevant treatments in this field, as well as the pathways involved in their action, relating this information to the previously described fibroid physiopathology, with the aim of better understanding their origin, to gain insight into their function and outlooks for their possible future use and in order to highlight ways that potential future therapies may be able to target the disease.

References

1. Commandeur AE, Styer AK, Teixeira JM (2015) Epidemiological and genetic clues for molecular mechanisms involved in uterine leiomyoma development and growth. Hum Reprod Update 21: 593.
2. Baird D, Dunson DB, Hill MC, Cousins D, Schectman JM (2003) High cumulative incidence of uterine leiomyoma in black and white women: Ultrasound evidence. Am J Obstet Gynecol 188: 100-107.
3. Cardozo ER, Clark AD, Banks NK, Henne MB, Stegmann BJ, et al. (2012) The estimated annual cost of uterine leiomyomata in the United States. Am J Obstet Gynecol 206: 211.e9.
4. Stewart EA (2001) Uterine fibroids. Lancet 357: 293-298.
5. Faerstein E, Szklo M, Rosenshein N (2001) Risk factors for uterine leiomyoma: A practice-based case-control study. I. African-American heritage, reproductive history, body size and smoking. Am J Epidemiol 153: 1-10.
6. Kim JJ, Kurita T, Bulun SE (2013) Progesterone action in endometrial cancer, endometriosis, uterine fibroids and breast cancer. Endocr Rev 34: 130–162.
7. Faerstein E, Szklo M, Rosenshein NB (2001) Risk factors for uterine leiomyoma: A practice-based case-control study. II. Atherogenic risk factors and potential sources of uterine irritation. Am J Epidemiol 153: 11-19.

8. Terry KL, De Vivo I, Hankinson SE, Missmer SA (2010) Reproductive characteristics and risk of uterine leiomyoma. Fertil Steril 94: 2703-2707.

9. Peddada SD, Laughlin SK, Miner K, Guyon J-P, Haneke K, et al. (2008) Growth of uterine leiomyomata among premenopausal black and white women. Proc Natl Acad Sci 105: 19887-19892.

10. Cesen-Cummings K, Houston KD, Copland JA, Moorman VJ, Walker CL, et al. (2003) Uterine leiomyomas express myometrial contractile-associated protein involved in pregnancy-related hormone signaling. J Soc Gynecol Investing 10: 11-20.

11. Cai YR, Dia XL, Wang SF, Zhang W, Zhang HT, et al. (2007) X-chromosomal inactivation analysis of uterine leiomyomas reveals a common clonal origin of different tumor nodules in some multiple leiomyomas. Int J Oncol 31: 1379-1389.

12. Holdsworth-Carson SJ, Zaitseva M, Vollenhoven BJ, Rogers PAW (2014) Clonality of smooth muscle and fibroblast cell populations isolated from human fibroid and myometrial tissues. Mol Hum Reprod 20: 250-259.

13. Ono M, Maruyama T, Masuda H, Kajitani T, Nagashima T, et al. (2007) Side population in human uterine myometrium displays phenotypic and functional characteristics of myometrial stem cells. Proc Natl Acad Sci 104: 18700-18705.

14. Ono M, Qiang W, Serna VA, Yin P, Coon JS 5th, et al. (2012) Role of stem cells in human uterine leiomyoma growth. PLoS ONE 7: e36935.

15. Fujii S, Suzuki A, Matsumura N, Kanamori T, Shime H, et al. (2004) Fibroids: basic science and etiology. International Congress Series. Amsterdam: Elsevier.

16. Islam MS, Protic O, Stortoni P, Grechi G, Lamanna P, et al. (2013) Complex networks of multiple factors in the pathogenesis of uterine leiomyoma. Fertil Steril 100: 178-193.

17. Nibert M, Heim S (1990) Uterine leiomyoma cytogenetics. Genes Chromosomes Cancer 2: 3-13.

18. Rein MS, Friedman AJ, Barbieri RL, Pavelka K, Fletcher JA, et al. (1991) Cytogenetic abnormalities in uterine leiomyomata. Obstet Gynecol 77: 923-926.

19. Ligon AH, Morton CC (2001) Leiomyomata: Heritability and cytogenetic studies. Hum Reprod Update 7: 8-14.

20. Sandberg AA (2005) Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: Leiomyoma. Cancer Genet Cytogenet 158: 1-26.

21. Mehine M, Kaasinen E, Mäkinen N, Katainen R, Kämpjärvi K, et al. (2013) Characterization of uterine leiomyomas by whole-genome sequencing. N Engl J Med 369: 43-53.

22. Kataoka S, Yamada H, Hoshi N, Kudo M, Hareyama H, et al. (2003) Cytogenetic analysis of uterine leiomyoma: The size, histopathology and GnRHa-response in relation to chromosome karyotype. Eur J Obstet Gynecol Reprod Biol 110: 58-62.

23. Sato F, Mori M, Nishi M, Kudo R, Miyake H (2002) Familial aggregation of uterine myomas in Japanese women. J Epidemiol 12: 249-253.

24. Luoto R, Kaprio J, Rutanen EM, Taipale P, Perola M, et al. (2000) Heritability and risk factors of uterine fibroids--The Finnish twin cohort study. Maturitas 37: 15-26.

25. Kashtan CE (1997) Alport syndrome. An inherited disorder of renal, ocular and cochlear basement membranes. Medicine 78: 338-360.

26. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG (2003) Alport's syndrome, Good pasture's syndrome and type IV collagen. N Engl J Med 348: 2543-2556.

27. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism and function. Cell 116: 281-297.

28. Carletti MZ, Christenson LK (2009) MicroRNA in the ovary and female reproductive tract. J Anim Sci 87: E29-38.

29. Chuang TD, Luo X, Panda H, Chegini N (2012) miR-93/106b and their host gene, MCM7, are differentially expressed in leiomyomas and functionally target F3 and IL-8. Mol Endocrinol 26: 1028-1042.

30. Bulun SE (2013) Uterine fibroids. N Engl J Med 369: 1344-1355.

31. Karmon AE, Cardozo ER, Rueda BR, Styer AK (2014) microRNAs in the development and pathobiology of uterine leiomyomata: does evidence support future strategies for clinical intervention? Hum Reprod Update 20: 670-687.

32. Wang T, Zhang X, Obijuru L, Laser J, Aris V, et al. (2007) A micro-RNA signature associated with race, tumor size and target gene activity in human uterine leiomyomas. Genes Chromosomes Cancer 46: 336-347.

33. Thompson CM, Koleske AJ, Chao DM, Young RA (1993) A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell 73: 1361-1375.

34. de Graaff MA, Cleton-Jansen AM, Szuhai K, Bovee JV (2013) Mediator complex subunit 12 exon 2 mutation analysis in different subtypes of smooth muscle tumors confirms genetic heterogeneity. Hum Pathol 44: 1597-1604.

35. Schoenmakers EF, Wanschura S, Mols R, Bullerdiek J, Van den Berghe H, et al. (1995) Recurrent rearrangements in the high-mobility group protein gene, HMGI-C, in benign mesenchymal tumours. Nat Genet 10: 436-444.

36. Schoenmakers EF, Huysmans C, Van de Ven WJ (1999) Allelic knockout of novel splice variants of human recombination repair gene RAD51B in t(12;14) uterine leiomyomas. Cancer Res 59: 19-23.

37. Quade BJ, Weremowicz S, Neskey DM, Vanni R, Ladd C, et al. (2003) Fusion transcripts involving HMGA2 are not a common molecular mechanism in uterine leiomyomata with rearrangements in 12q15. Cancer Res 63: 1351-1358.

38. Pérot G, Croce S, Ribeiro A, Lagarde P, Velasco V, et al. (2012) MED12 alterations in both human benign and malignant uterine soft tissue tumors. PLoS ONE 7: e40015.

39. Copley MR, Babovic S, Benz C, Knapp DJ, Beer PA, et al. (2013) The Lin28b-let-7-Hmga2 axis determines the higher self-renewal potential of fetal haematopoietic stem cells. Nat Cell Biol 15: 916-925.

40. Hodge JC, Kim TM, Dreyfuss JM, Somasundaram P, Christacos NC, et al. (2012) Expression profiling of uterine leiomyomata cytogenetic subgroups reveals distinct signatures in matched myometrium: Transcriptional profiling of the t(12;14) and evidence in support of predisposing genetic heterogeneity. Hum Mol Genet 21: 2312-2329.

41. Helmke BM, Markowski DN, Müller MH, Sommer A, Müller J, et al. (2011) HMGA proteins regulate the expression of FGF2 in uterine fibroids. Mol Hum Reprod 17: 135-142.

42. Marshall LM, Spiegelman D, Barbieri RL, Goldman MB, Manson JE, et al. (1997) Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. Obstet Gynecol 90: 967-973.

43. Baird DD, Hill MC, Schectman JM, Hollis BW (2013) Vitamin d and the risk of uterine fibroids. Epidemiology 24: 447-453.

44. Moorman PG, Leppert P, Myers ER, Wang F (2013) Comparison of characteristics of fibroids in African American and white women undergoing premenopausal hysterectomy. Fertil Steril 99: 768-776.

45. Marsh EE, Ekpo GE, Cardozo ER, Brocks M, Dune T, et al. (2013) Racial differences in fibroid prevalence and ultrasound findings in asymptomatic young women (18-30 years old): A pilot study. Fertil Steril 99: 1951-1957.

46. Susanna D Mitro, Ami R Zota (2015) Vitamin D and uterine leiomyoma among a sample of US women: Findings from NHANES, 2001-2006. Reprod Toxicol (Elmsford, N.Y.) 57: 81-86.

47. Ishikawa H, Reierstad S, Demura M, Rademaker AW, Kasai T, et al. (2009) High aromatase expression in uterine leiomyoma tissues of African-American women. J Clin Endocrinol Metab 94: 1752-1756.

48. Davis BJ, Risinger JI, Chandramouli GV, Bushel PR, Baird DD, et al. (2013) Gene expression in uterine leiomyoma from tumors likely to be growing (from black women over 35) and tumors likely to be non-growing (from white women over 35). PLoS ONE 8: e63909.

49. Al-Hendy A, Salama SA (2006) Catechol-O-methyltransferase polymorphism is associated with increased uterine leiomyoma risk in different ethnic groups. J Soc Gynecol Investig 13: 136-144.

50. Feng Y, Zhao X, Zhou C, Yang L, Liu Y, et al. (2013) The associations between the Val158Met in the catechol-O-methyltransferase (COMT) gene and the risk of uterine leiomyoma (ULM). Gene 529: 296-299.

51. Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, et al. (2002) Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: Third National Health and Nutrition Examination Survey, 1988-1994. Am J Clin Nutr 76: 187-192.

52. Paffoni A, Somigliana E, Vigano P, Benaglia L, Cardellicchio L, et al. (2013) Vitamin D status in women with uterine leiomyomas. J Clin Endocrinol Metab 98: E1374-1378.

53. Sabry M, Halder SK, Allah AS, Roshdy E, Rajaratnam V, et al. Serum vitamin D3 level inversely correlates with uterine fibroid volume in different ethnic groups: a cross-sectional observational study. Int J Womens Health 5: 93-100.

54. Zhao G, Ford ES, Tsai J, Li C, Croft JB (2012) Factors associated with vitamin D deficiency and inadequacy among women of child-bearing age in the United States. ISRN Obstet Gynecol 2012: 691486.

55. Brakta S, Diamond JS, Al-Hendy A, Diamond MP, Halder SK (2015) Role of vitamin D in uterine fibroid biology. Fertil Steril 104: 698-706.

56. Bläuer M1, Rovio PH, Ylikomi T, Heinonen PK (2009) Vitamin D inhibits myometrial and leiomyoma cell proliferation *in vitro*. Fertil Steril 91: 1919-1925.

57. Halder SK, Sharan C, Al-Hendy A (2012) 1,25-dihydroxyvitamin D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model. Biol Reprod 86: 116.

58. Sharan C, Halder SK, Thota C, Jaleel T, Nair S, et al. (2011) Vitamin D inhibits proliferation of human uterine leiomyoma cells via catechol-O-methyltransferase. Fertil. Steril. 95: 247–253.

59. Borahay MA, Al-Hendy A, Kilic GS, Boehning D (2015) Signaling pathways in leiomyoma: Understanding pathobiology and implications for therapy. Mol Med (Cambridge, Mass) 21: 242.

60. Zadshir A, Tareen N, Pan D, Norris K, Martins D (2005) The prevalence of hypovitaminosis D among US adults: Data from the NHANES III. Ethn Dis 15: S5-97-101.

61. Halder SK, Osteen KG, Al-Hendy A (2013) 1,25-dihydroxyvitamin d3 reduces extracellular matrix-associated protein expression in human uterine fibroid cells. Biol Reprod 89: 150.

62. Al-Hendy A, Diamond MP, El-Sohemy A, Halder SK (2015) 1,25-Dihydroxyvitamin D3 regulates expression of sex steroid receptors in human uterine fibroid cells. J Clin Endocrinol Metab 100: E572-582.

63. Sozen I, Arici A (2002) Interactions of cytokines, growth factors and the extracellular matrix in the cellular biology of uterine leiomyomata. Fertil Steril 78: 1-12.

64. Lemmon MA, Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. Cell 141: 1117-1134.

65. Yu L, Saile K, Swartz CD, He H, Zheng X, et al. (2008) Differential expression of receptor tyrosine kinases (RTKs) and IGF-I pathway activation in human uterine leiomyomas. Mol Med 14: 264-275.

66. Kolch W (2000) Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. Biochem J 2: 289-305.

67. Lopez GN, Turck CW, Schaufele F, Stallcup MR, Kushner PJ (2001) Growth factors signal to steroid receptors through mitogen-activated protein kinase regulation of p160 co-activator activity. J Biol Chem 276: 22177-22182.

68. Wong CW, McNally C, Nickbarg E, Komm BS, Cheskis BJ (2002) Estrogen receptor-interacting protein that modulates its non-genomic activity-crosstalk with Src/Erk phosphorylation cascade. Proc Natl Acad Sci U S A 99: 14783-14788.

69. Fayed YM, Tsibris JC, Langenberg PW, Robertson AL Jr (1989) Human uterine leiomyoma cells: binding and growth responses to epidermal growth factor, platelet-derived growth factor and insulin. Lab Invest 60: 30-37.

70. Rossi MJ, Chegini N, Masterson BJ (1992) Presence of epidermal growth factor, platelet-derived growth factor, and their receptors in human myometrial tissue and smooth muscle cells: Their action in smooth muscle cells *in vitro*. Endocrinology 130: 1716-1727.

71. Arici A, Sozen I (2003) Expression, menstrual cycle-dependent activation and bimodal mitogenic effect of transforming growth factor-beta1 in human myometrium and leiomyoma. Am J Obstet Gynecol 188: 76-83.

72. Liang M, Wang H, Zhang Y, Lu S, Wang Z (2006) Expression and functional analysis of platelet-derived growth factor in uterine leiomyomata. Cancer Biol Ther 5: 28-33.

73. Arici A, Sozen I (2000) Transforming growth factor-beta3 is expressed at high levels in leiomyoma where it stimulates fibronectin expression and cell proliferation. Fertil Steril 73: 1006-1011.

74. Lee BS, Nowak RA (2001) Human leiomyoma smooth muscle cells show increased expression of transforming growth factor-beta 3 (TGF beta 3) and altered responses to the anti-proliferative effects of TGF beta. J Clin Endocrinol Metab 86: 913-920.

75. Di Lieto A, De Falco M, Staibano S, Iannotti F, Scaramellino M, et al. (2003) Effects of gonadotropin releasing hormone agonists on uterine volume and vasculature and on the immunohistochemical expression of basic fibroblast growth factor (bFGF) in uterine leiomyomas. Int J Gynecol Pathol 22: 353-358.

76. Maruo T, Ohara N, Wang J, Matsuo H (2004) Sex steroidal regulation of uterine leiomyoma growth and apoptosis. Hum Reprod Update 10: 207-220.

77. Burroughs KD, Howe SR, Okubo Y, Fuchs-Young R, LeRoith D, et al. (2002) Dysregulation of IGF-I signaling in uterine leiomyoma. J Endocrinol 172: 83-93.

78. Peng L, Wen Y, Han Y, Wei A, Shi G, et al. (2009) Expression of insulin-like growth factors (IGFs) and IGF signaling: molecu-lar complexity in uterine leiomyomas. Fertil Steril 91: 2664-2675.

79. Gentry CC, Okolo SO, Fong LF, Crow JC, Maclean AB, et al. (2001) Quantification of vascular endothelial growth factor-A in leiomyomas and adjacent myometrium. Clin Sci (Lond) 101: 691-695.

80. Xu Q, Ohara N, Chen W, Liu J, Sasaki H, et al. (2006) Progesterone receptor modulator CDB-2914 down-regulates vascular endothelial growth factor, adrenomedullin and their receptors and modulates progesterone receptor content in cultured human uterine leiomyoma cells. Hum Reprod 21: 2408-2416.

81. Hassan MH, Eduardo E, Hossam MMA, Farid MAH, Salama AS, et al. (2008) Memy I: A novel murine model for uterine leiomyoma using adenovirus-enhanced human fibroid explants in severe combined immune deficiency mice. Am J Obstet Gynecol 199: 151-158.

82. Dinarello CA (2007) Historical insights into cytokines. Eur J Immunol 37: S34-45.

83. Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A (2006) IL-13 signaling through the IL-13 alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. Nat Med 12: 99-106.

84. Chen Q, Rabach L, Noble P, Zheng T, Lee CG, et al. (2005) IL-11 receptor alpha in the pathogenesis of IL-13-induced inflammation and remodeling. J Immunol 174: 2305-2313.

85. Pietrowski D, Thewes R, Sator M, Denschlag D, Keck C, et al. (2009) Uterine leiomyoma is associated with a polymorphism in the interleukin 1-beta gene. Am J Reprod Immunol 62: 112-117.

86. Hsieh YY, Chang CC, Tsai CH, Lin CC, Tsai FJ (2007) Interleukin (IL)-12 receptor beta1 codon 378G homozygote and allele, but not IL-1 (beta-511 promoter, 3953 exon 5, receptor antagonist), IL-2 114, IL-4 590 intron 3, IL-8 3'-UTR 2767 and IL-18 105, are associated with higher susceptibility to leiomyoma. Fertil Steril 87: 886-895.

87. Fujita M (1985) Histological and biochemical studies of collagen in human uterine leiomyomas. Hokkaido Igaku Zasshi 60: 602-615.

88. Gelse K, Pöschl E, Aigner T (2003) Collagens--structure, function and biosynthesis. Adv Drug Deliv Rev 55: 1531-1546.

89. Malik M, Norian J, McCarthy-Keith D, Britten J, Catherino WH (2010) Why leiomyomas are called fibroids: The central role of extracellular matrix in symptomatic women. Semin Reprod Med 28: 169-179.

90. Catherino WH, Leppert PC, Stenmark MH, Payson M, Potlog-Nahari C, et al. (2004) Reduced dermatopontin expression is a molecular link between uterine leiomyomas and keloids. Genes Chromosomes Cancer 40: 204-217.

91. Arici A, Sozen I (2000) Transforming growth factor-beta3 is expressed at high levels in leiomyoma where it stimulates fibronectin expression and cell proliferation. Fertil Steril 73: 1006-1011.

92. Tanwar PS, Lee HJ, Zhang L, Zukerberg LR, Taketo MM, et al. (2009) Constitutive activation of beta-catenin in uterine stroma and smooth muscle leads to the development of mesenchymal tumors in mice. Biol Reprod 81: 545-552.

93. Markowski DN, Bartnitzke S, Löning T, Drieschner N, Helmke BM, et al. (2012) MED12 mutations in uterine fibroids--their relationship to cytogenetic subgroups. Int J Cancer 131: 1528-1536.

94. Ono M, Yin P, Navarro A, Moravek MB, Coon JS, et al. (2013) Paracrine activation of WNT/Î²-catenin pathway in uterine leiomyoma stem cells promotes tumor growth. Proc Natl Acad Sci U S A 110: 17053-17058.

95. Niederreither K, Dollé P (2008) Retinoic acid in development: towards an integrated view. Nat Rev Genet 9: 541-553.

96. Nam DH, Ramachandran S, Song DK, Kwon KY, Jeon DS, et al. (2007) Growth

inhibition and apoptosis induced in human leiomyoma cells by treatment with the PPAR gamma ligand ciglitizone. Mol Hum Reprod 13: 829-836.

97. Lethaby A, Vollenhoven B, Sowter M. (2001) Pre- operative GnRH analogue therapy before hysterectomy or myomectomy for uterine fibroids. Cochrane Database Syst Rev CD000547.

98. Maruo T1, Ohara N, Wang J, Matsuo H (2004) Sex steroidal regulation of uterine leiomyoma growth and apoptosis. Hum Reprod Update 10: 207-220.

99. Sadan O, van Iddekinge B, van Gelderen CJ, Savage N, Becker PJ, et al. (1987) Oestrogen and progesterone receptor concentrations in leiomyoma and normal myometrium. Ann Clin Biochem 24: 263-267.

100. Otsuka H, Shinohara M, Kashimura M, Yoshida K, Okamura Y (1989) A comparative study of the estrogen receptor ratio in myometrium and uterine leiomyomas. Int J Gynaecol Obstet 29: 189-194.

101. Olmos Grings A, Lora V, Dias Ferreira G, Simoni Brum I, von Eye Corleta H, et al. (2012) Protein expression of estrogen receptors a and b and aromatase in myometrium and uterine leiomyoma. Gynecol Obstet Invest 73: 113–117.

102. Prossnitz ER, Arterburn JB, Smith HO, Oprea TI, Sklar LA, et al. (2008) Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. Annu Rev Physiol 70: 165-190.

103. Ishikawa H, Ishi K, Serna VA, Kakazu R, Bulun SE, et al. (2010) Progesterone is essential for maintenance and growth of uterine leiomyoma. Endocrinology 151: 2433-2442.

104. Gao Z, Matsuo H, Nakago S, Kurachi O, Maruo T (2002) p53 Tumor suppressor protein content in human uterine leiomyomas and its down-regulation by 17 beta-estradiol. J Clin Endocrinol Metab 87: 3915-3920.

105. Barbarisi A, Petillo O, Di Lieto A, Melone MA, Margarucci S, et al. (2001) 17-beta estradiol elicits an autocrine leiomyoma cell proliferation: Evidence for a stimulation of protein kinase-dependent pathway. J Cell Physiol 186: 414-424.

106. Ciarmela P, Bloise E, Gray PC, Carrarelli P, Islam MS, et al. (2011) Activin-A and myostatin response and steroid regulation in human myometrium: Disruption of their signalling in uterine fibroid. J Clin Endocrinol Metab 96: 755-765.

107. Benassayag C, Leroy MJ, Rigourd V, Robert B, Honoré JC, et al. (1999) Estrogen receptors (ERalpha/ERbeta) in normal and pathological growth of the human myometrium: Pregnancy and leiomyoma. Am J Physiol 276: E1112-1118.

108. Hermon TL, Moore AB, Yu L, Kissling GE, Castora FJ, et al. (2008) Estrogen receptor alpha (ERalpha) phospho-serine-118 is highly expressed in human uterine leiomyomas compared to matched myometrium. Virchows Arch 453: 557-569.

109. Andersen J, Dyreyes VM, Barbieri RL, Coachman DM, Miksicek RJ (1995) Leiomyoma primary cultures have elevated transcriptional response to estrogen compared with autologous myometrial cultures. J Soc Gynecol Investig 2: 542-551.

110. Pedeutour F, Quade BJ, Weremowicz S, Dal Cin P, Ali S, et al. (1998) Localization and expression of the human estrogen receptor beta gene in uterine leiomyomata. Genes Chromosomes Cancer 23: 361-366.

111. Deng L, Wu T, Chen XY, Xie L, Yang J. (2012) Selective estrogen receptor modulators (SERMs) for uterine leiomyomas. Cochrane Database Syst Rev 10: CD005287.

112. Shozu M, Murakami K, Inoue M (2004) Aromatase and leiomyoma of the uterus. Semin Reprod Med 22: 51-60.

113. Sumitani H, Shozu M, Segawa T, Murakami K, Yang HJ, et al. (2000) *In situ* estrogen synthesized by aromatase P450 in uterine leiomyoma cells promotes cell growth probably via an autocrine/intracrine mechanism. Endocrinology 141: 3852-3861.

114. Lamminen S, Rantala I, Helin H, Rorarius M, Tuimala R (1992) Proliferative activity of human uterine leiomyoma cells as measured by automatic image analysis. Gynecol Obstet Invest 34: 111-114.

115. Ishikawa H, Ishi K, Serna VA, Kakazu R, Bulun SE, et al. (2010) Progesterone is essential for maintenance and growth of uterine leiomyoma. Endocrinology 151: 2433-2442.

116. Arici A, Sozen I (2000) Transforming growth factor-beta3 is expressed at high levels in leiomyoma where it stimulates fibronectin expression and cell proliferation. Fertil Steril 73: 1006-1011.

117. Maruo T, Matsuo H, Shimomura Y, Kurachi O, Gao Z, et al. (2003) Effects of progesterone on growth factor expression in human uterine leiomyoma. Steroids 68: 817-824.

118. Yamada T, Satoshi N, Osamu K, Jiayin W, Shigeki T, et al. (2004) Progesterone down-regulates insulin-like growth factor-I expression in cultured human uterine leiomyoma cells. Hum Reprod 19: 815-821.

119. Matsuo H, Maruo T, Samoto T (1997) Increased expression of Bcl-2 protein in human uterine leiomyoma and its up-regulation by progesterone. J Clin Endocrinol Metab 82: 293-299.

120. Shimomura Y, Matsuo H, Samoto T, Maruo T (1998) Up-regulation by progesterone of proliferating cell nuclear antigen and epidermal growth factor expression in human uterine leiomyoma. J Clin Endocrinol Metab 83: 2192-2198.

121. Maruo T, Matsuo H, Samoto T, Shimomura Y, Kurachi O, et al. (2000) Effects of progesterone on uterine leiomyoma growth and apoptosis. Steroids 65: 585-592.

122. Tiltman AJ (1985) The effect of progestins on the mitotic activity of uterine fibromyomas. Int J Gynecol Pathol 4: 89-96.

123. Kawaguchi K, Fujii S, Konishi I, Nanbu Y, Nonogaki H, et al. (1989) Mitotic activity in uterine leiomyomas during the menstrual cycle. Am J Obstet Gynecol 160: 637-641.

124. Lamminen S, Rantala I, Helin H, Rorarius M, Tuimala R (1992) Proliferative activity of human uterine leiomyoma cells as measured by automatic image analysis. Gynecol Obstet Invest 34: 111-114.

125. Rosati P, Exacoustòs C, Mancuso S (1992) Longitudinal evaluation of uterine myoma growth during pregnancy. A sonographic study. J Ultrasound Med 11: 511-515.

126. Neiger R, Sonek JD, Croom CS, Ventolini G (2006) Pregnancy-related changes in the size of uterine leiomyomas. J Reprod Med 51: 671-674.

127. Hanahan D, Coussens LM (2012) Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 21: 309-322.

128. Marelli G, Codegoni AM, Bizzi A (1989) Estrogen and progesterone receptors in leiomyomas and normal uterine tissues during reproductive life. Acta Eur Fertil 20: 19-22.

129. Brandon DD, Bethea CL, Strawn EY, Novy MJ, Burry KA, et al. (1993) Progesterone receptor messenger ribonucleic acid and protein are over-expressed in human uterine leiomyomas. Am J Obstet Gynecol 169: 78-85.

130. Viville B, Charnock-Jones DS, Sharkey AM, Wetzka B, Smith SK (1997) Distribution of the A and B forms of the progesterone receptor messenger ribonucleic acid and protein in uterine leiomyomata and adjacent myometrium. Hum Reprod 12: 815-822.

131. Ying Z, Weiyuan Z (2009) Dual actions of progesterone on uterine leiomyoma correlate with the ratio of progesterone receptor A: B. Gynecol Endocrinol 25: 520-523.

132. Fujimoto J, Hirose R, Ichigo S, Sakaguchi H, Li Y, et al. (1998) Expression of progesterone receptor form A and B mRNAs in uterine leiomyoma. Tumour Biol 19: 126-131.

133. Boonyaratanakornkit V, Edwards DP (2007) Receptor mechanisms mediating non-genomic actions of sex steroids. Semin Reprod Med 25: 139-153.

134. Condon JC, Hardy DB, Kovaric K, Mendelson CR (2006) Up-regulation of the progesterone Franceschi S. The IARC commitment to cancer prevention: the example of receptor (PR)-C isoform in laboring myometrium by activation of nuclear factor-kappaB may contribute to the onset of labor through inhibition of PR function. Mol Endocrinol 20: 764-775.

135. Merlino AA, Welsh TN, Tan H, Yi LJ, Cannon V, et al. (2007) Nuclear progesterone receptors in the human pregnancy myometrium: Evidence that parturition involves functional progesterone withdrawal mediated by increased expression of progesterone receptor-A. J Clin Endocrinol Metab 92: 1927-1933.

136. Patel B, Elguero S, Thakore S, Dahoud W, Bedaiwy M, et al. (2015) Role of nuclear progesterone receptor isoforms in uterine pathophysiology. Hum Reprod Update 21: 155-173.

137. Donnez J, Dolmans MM (2016) Uterine fibroid management: from the present to the future. Hum Reprod Update 22: 665-686.

138. Khan AT, Shehmar M, Gupta JK (2014) Uterine fibroids: current perspectives. Int J Womens Health 6: 95.

139. Donnez J, Schrurs B, Gillerot S, Sandow J, Clerckx F (1989) Treatment of uterine fibroids with implants of gonadotropin-releasing hormone agonist: Assessment by hysterography. Fertil Steril 51: 947-950.

140. Bozzini N, Rodrigues CJ, Petti DA (2003) Effects of treatment with gonadotropin releasing hormone agonist on the uterine leiomyomata structure. Acta Obstet Gynecol Scand 82: 330–334.

141. Rutgers JL, Spong CY, Sinow R, Heiner J (1995) Leuprolide acetate treatment and myoma arterial size. Obstet Gynecol 86: 386-388.

142. Hoellen F, Griesinger G, Bohlmann MK (2013) Therapeutic drugs in the treatment of symptomatic uterine fibroids. Expert Opin Pharmacother 14: 2079-2085.

143. Moroni RM, Martins WP, Ferriani RA, Vieira CS, Nastri CO, et al. (2015) Add-back therapy with GnRH analogues for uterine fibroids. Cochrane Database Syst Rev CD010854.

144. Kamath MS, Kalampokas EE, Kalampokas TE (2014) Use of GnRH analogues pre-operatively for hysteroscopic resection of submucous fibroids: A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol 177: 11-18.

145. Bizzarri N, Ghirardi V, Remorgida V, Venturini PL, Ferrero S (2015) Three-month treatment with triptorelin, letrozole and ulipristal acetate before hysteroscopic resection of uterine myomas: prospective comparative pilot study. Eur J Obstet Gynecol Reprod Biol 192: 22-26.

146. Fiscella K, Eisinger SH, Meldrum S, Feng C, Fisher SG, et al. (2006) Effect of mifepristone for symptomatic leiomyomata on quality of life and uterine size: a randomized controlled trial. Obstet Gynecol 108: 1381-1387.

147. Donnez J, Tatarchuk TF, Bouchard P, Puscasiu L, Zakharenko NF, et al. (2012) Ulipristal acetate versus placebo for fibroid treatment before surgery. N Engl J Med 366: 409-420.

148. Ohara N, Morikawa A, Chen W, Wang J, DeManno DA, et al. (2007) Comparative effects of SPRM asoprisnil (J867) on proliferation, apoptosis and the expression of growth factors in cultured uterine leiomyoma cells and normal myometrial cells. Reprod Sci 14: 20-27.

149. Donnez J, Tatarchuk TF, Bouchard P, Puscasiu L, Zakharenko NF, et al. (2012) Ulipristal acetate versus placebo for fibroid treatment before surgery. N Engl j Med 366: 409-420.

150. Donnez J, Tomaszewski J, Vázquez F, Bouchard P, Lemieszczuk B, et al. (2012) Ulipristal acetate versus leuprolide acetate for uterine fibroids. N Engl J Med 366: 421-432.

151. Courtoy G, Donnez J, Marbaix E, Dolmans MM (2015) In vivo mechanisms of uterine myoma volume reduction with ulipristal acetate treatment. Fertil Steril 104: 426-434.

152. Goyeneche AA, Seidel EE, Telleria CM (2012) Growth inhibition induced by anti-progestins RU-38486, ORG-31710 and CDB-2914 in ovarian cancer cells involves inhibition of cyclin dependent kinase 2. Invest New Drugs 30: 967-980.